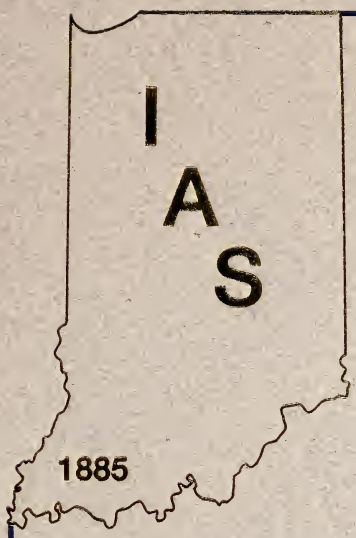

ISSN 0073-6767

Proceedings of the

Indiana Academy of Science

Volume 106 *Number 3-4*

The *PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE* is a quarterly journal dedicated to promoting scientific research and the diffusion of scientific information; to encouraging communication and cooperation among scientists; and to improving education in the sciences.

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PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

Founded 29 December 1885

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Volume 106 Number 3-4
1997

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Published at Bloomington, Indiana
May 12, 2000

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PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

Volume 106, No. 3-4 (1997)

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SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY OF THE ARACHNOID VILLI IN SQUIRREL MONKEY IN RELATION TO CEREBROSPINAL FLUID ABSORPTION

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ABSTRACT: The surface features and fine structure of arachnoid villi were examined using scanning (SEM) and transmission electron microscopy (TEM). With SEM, the endothelium of the superior sagittal sinus was seen extending over the surface of the villi as a continuous layer of endothelial cells that exhibited folds and crypts. The bulging luminal surface of the endothelial cells displayed slender processes, short microvilli, and pores of various sizes. Using TEM, the uninterrupted endothelial cells displayed slender interdigitating cytoplasmic processes that were joined by desmosomes. The cytoplasm of the endothelial cells contained numerous micropinocytotic vesicles and giant vacuoles. The giant vacuoles communicated with basal pinocytotic vesicles and surface pores, apparently creating transcellular channels in the endothelium. The endothelial covering separated the subendothelial space from the venous sinus. The cores contained arachnoid cells, fibroblasts, macrophages, and a network of anastomosing channels. The slender, overlapping cytoplasmic processes of the arachnoid cells lined the channels. The villi were devoid of endothelium-lined tubes and blood vessels. The shallow endothelial crypts seen in some villi were closed off from the subendothelial space by desmosomes. The villi were innervated by myelinated axons. The ultrastructural features of the arachnoid villi of the squirrel monkey revealed by this study are consistent with their function of CSF absorption by transcellular bulk flow and streaming through the surface pores into the dural venous sinuses.

KEYWORDS: Arachnoid cells, channels, desmosomes, endothelial cells, giant vacuoles, pores, vesicles.

INTRODUCTION

The arachnoid villi, or granulations, are small, blunt herniations of the arachnoid membrane which project into the cerebral veins and dural sinuses through small deficiencies in the dura mater. The villi play an essential role in the drainage and absorption of cerebrospinal fluid (CSF) into the venous sinuses. However, the mechanism by which CSF is transported across the villi into the venous blood is unclear.

The histology of the arachnoid villi was described by Weed (1914), Welch and Friedman (1960), Turner (1961), Millen and Woolam (1962), Jayatilaka (1965a), and Potts, *et al.* (1972). A number of ultrastructural studies were carried out on the arachnoid villi of various mammals (Jayatilaka, 1965b; Alksne

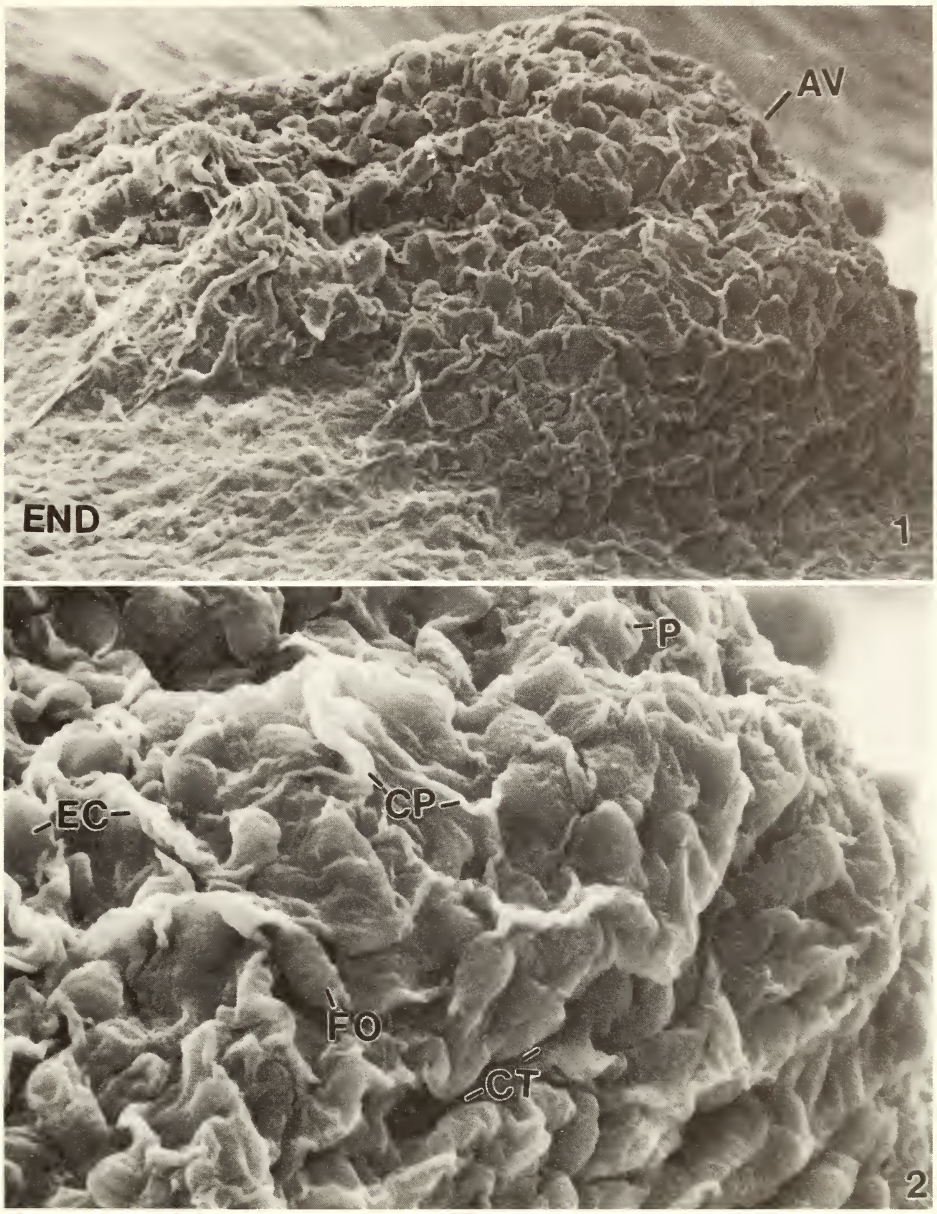
and White, 1965; Shabo and Maxwell, 1968a, b; Alksne and Lovings, 1972a, b; Gomez, *et al.,* 1973; Gomez and Potts, 1974; Gomez, *et al.,* 1974; Peters, *et al.,* 1976; Tripathi, 1973). These studies demonstrated that the arachnoid villi were invested by endothelial cells, that internally the villi were composed of arachnoid tissue and collagen bundles, and that the villi were traversed by a labyrinth of intercellular channels which communicated with the subarachnoid space around the brain. Some investigators asserted that the endothelium of the dural venous sinuses that invested the villi was continuous and joined by tight junctions, supporting Weed's (1923) "closed" system hypothesis which implied that CSF absorption took place across the endothelial covering of the arachnoid villi (Shabo and Maxwell, 1968a, b; Alksne and Lovings, 1972a, b). Other researchers showed that the villus core contained tubular channels lined by endothelium which was continuous with the lining of the dural sinuses, allowing direct flow of CSF from the subarachnoid space into the venous sinuses. Thus, an "open" system was proposed (Welch and Friedman, 1960; Welch and Pollay, 1961; Jayatilaka, 1965a, b; Hayes, *et al.,* 1971; Potts, *et al.,* 1972; Gomez and Potts, 1974; Gomez, *et al.,* 1974). Furthermore, Tripathi (1973) and Tripathi and Tripathi (1974) demonstrated that the giant vacuoles formed within the endothelial lining of monkey arachnoid villi allowed movement of CSF into the venous system by bulk flow.

Kida, *et al.* (1988) reported that the entire luminal surface of human arachnoid villi was invested by endothelial cells. However, other investigators showed that only a small portion of the human arachnoid villus is covered by endothelium, the rest is mainly invested with a layer of arachnoid cells (Upton and Weller, 1985; Yamashima, 1986). Studies by d'Avella, *et al.* (1980, 1983) on human arachnoid villi revealed the presence of giant intracellular vacuoles, pinocytotic vesicles, and large gaps between them in the endothelial cells, supporting both the "closed" and "open" mechanisms of CSF absorption. However, Upton and Weller (1985) did not observe any pores in the endothelium covering human arachnoid villi.

Thus, the mechanism of CSF absorption through the arachnoid villi into the venous sinuses still remains controversial. The aim of the present study is to elucidate the surface features and fine structure of the arachnoid villi in squirrel monkey and to relate them to the mechanism of CSF absorption.

MATERIALS AND METHODS

The squirrel monkey, *Saimiri sciureus*, used in the present study was obtained from the monkey colony maintained at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia. A normal adult squirrel monkey was anesthetized at the Histochemical Laboratory at the Yerkes Primate Research Center using an appropriate dose of sodium nembutal given intraperitoneally and was perfused with 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 for 30 minutes. After craniotomy, twelve arachnoid villi were excised from the inner wall of the superior sagittal sinus under a dissecting microscope and



Figures 1 and 2. **Figure 1.** A scanning electron micrograph (SEM) of an arachnoid villus (AV) projecting into the lumen of the superior sagittal sinus. Note the continuity of the endothelial lining (END) of the sinus with that of the villus (675X). **Figure 2.** An SEM of the same villus shown in Figure 1 (1,500X). Note the somewhat rounded profiles of the endothelial cells (EC) with cytoplasmic processes (CP), crypts (CT), folds (FO), and pores (P).

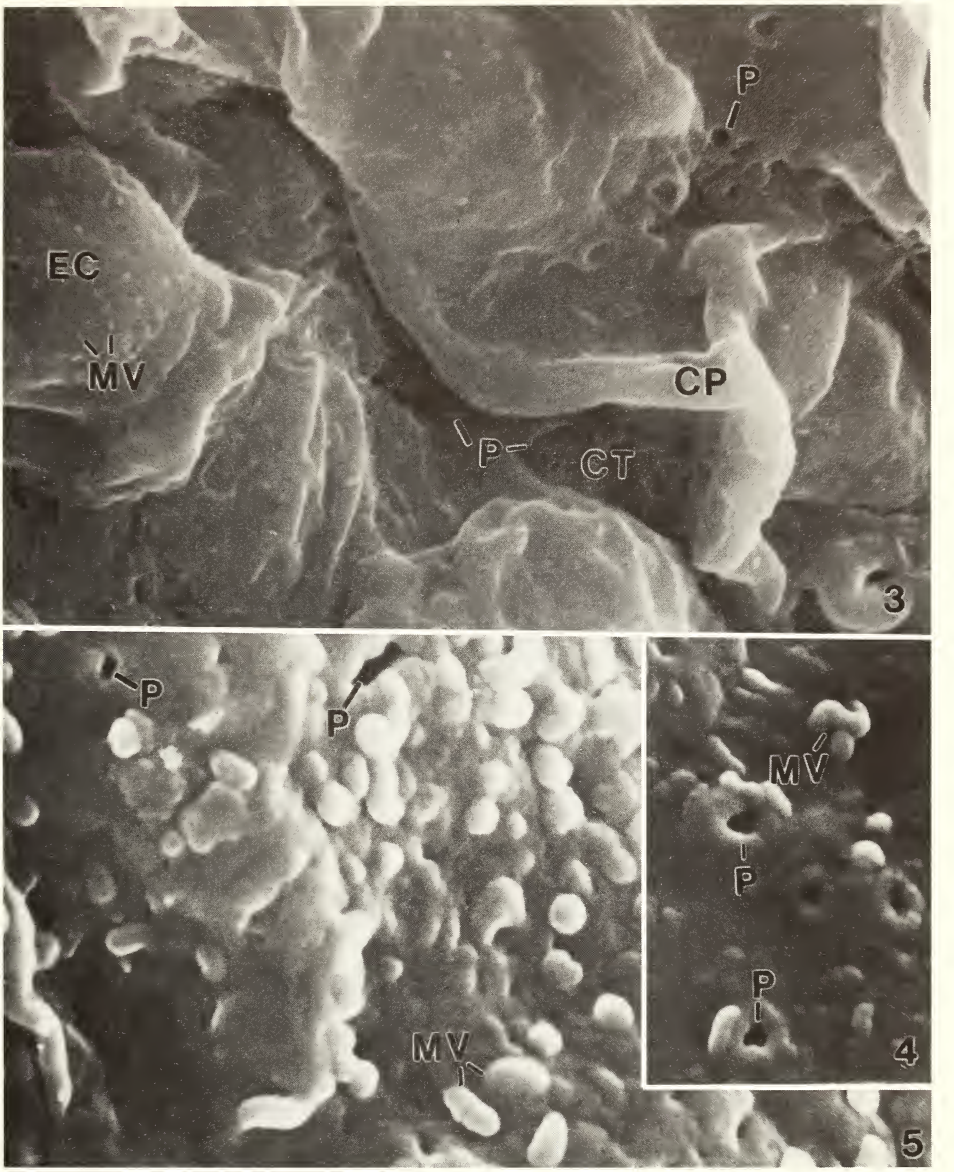
fixed in fresh 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for two hours at room temperature. The samples were rinsed in phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer. The material was dehydrated in an ethanol series, transferred to propylene oxide, and embedded in Epon 812 (Luft, 1961). Polymerization was carried out overnight at 60° C. The sections were cut on a Porter-Blum MT_{-2} ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an RCA-3C and a Hitachi HU-11A transmission electron microscope (TEM). Similarly fixed material was dried using the liquid CO_2 critical point method, coated with gold/palladium, and examined in an ETEC autoscan scanning electron microscope (SEM). Two (2) μm thick sections were cut using glass knives, stained with azure II, and examined under a light microscope (LM).

RESULTS

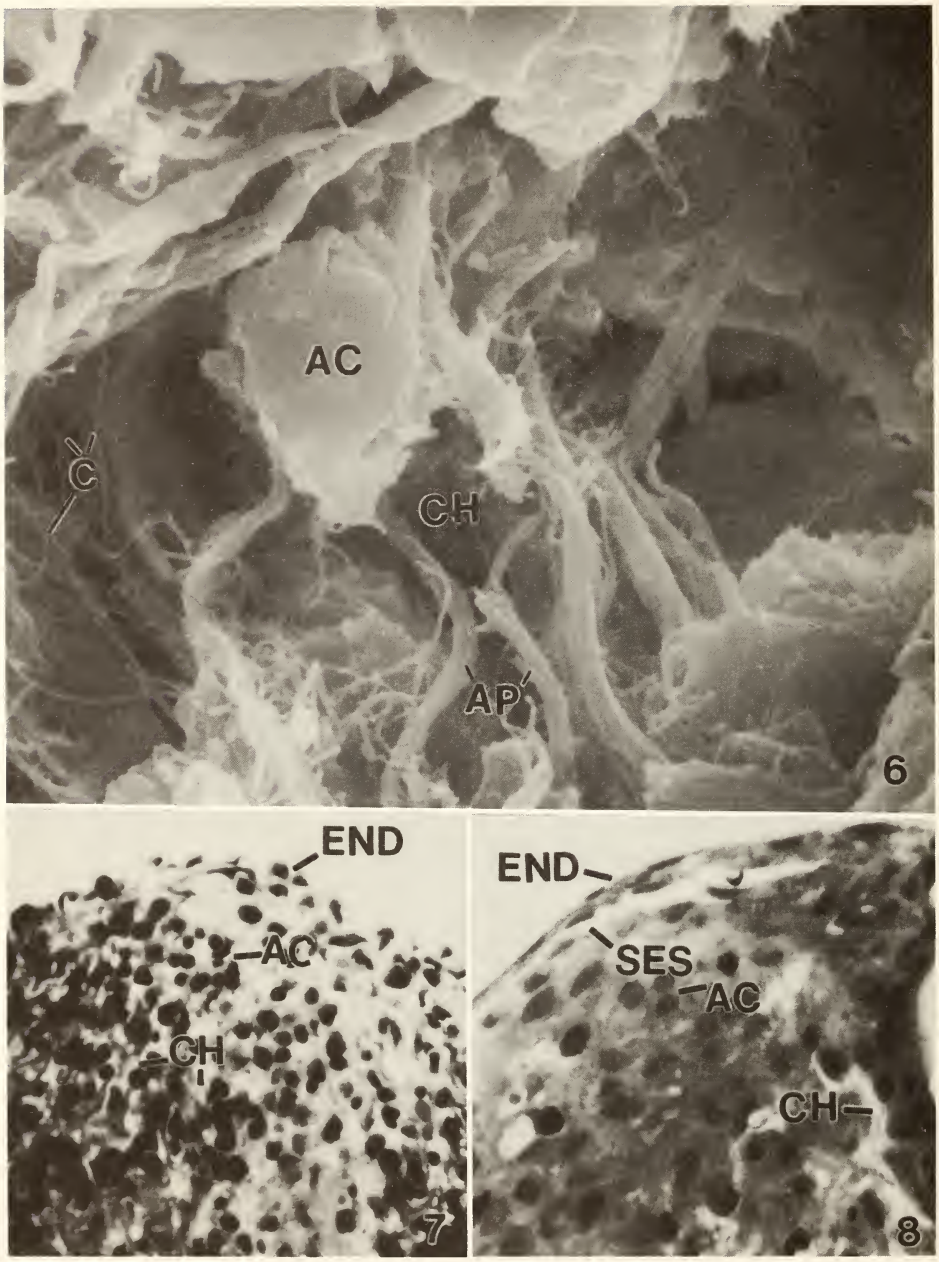
Scanning Electron Microscopy. After opening the dorsal wall of the superior sagittal sinus, many arachnoid villi measuring 125-200 μm in diameter were seen protruding into the sinus lumen. A panoramic view of the inner wall of the sinus revealed that the intact endothelial lining of the superior sagittal sinus extended over the villi to form their endothelial covering (Figure 1). The endothelial covering displayed folds and crypts. The endothelial cells appeared somewhat rounded in contour, possibly due to the presence of underlying giant vacuoles and/or nuclei (Figure 2). The cells had slender cytoplasmic processes and displayed short, club-shaped microvilli on their luminal surface (Figures 3-5). A prominent surface feature of the endothelial cells was the presence of pores measuring about 0.4 μm in diameter with somewhat thickened margins. More pores were found at the attenuated periphery than in the central region of the endothelial cells (Figures 3-5). Longitudinal sections through the middle of the villi revealed that the central core contained arachnoid cells with stout cytoplasmic processes and channels containing collagen bundles (Figure 6).

Light and Transmission Electron Microscopy. In semi-thin sections, the arachnoid villi were composed of a continuous, thin endothelial covering and an underlying core of arachnoid cells and interconnecting channels (Figures 7 and 8). The arachnoid villi were devoid of blood vessels, and they lacked the endothelium-lined tubes which have been reported in the arachnoid villi of other animals (Figures 7 and 8: Jayatilaka, 1965a; Potts, *et al.,* 1972). A pictorial summary of the general organization of the arachnoid villi of the squirrel monkey as revealed by electron microscopy is presented in Figure 9.

Transmission electron micrographs of the arachnoid villi revealed that their continuous endothelial covering was composed of fusiform endothelial cells displaying short microvilli on their luminal surface. The cells were separated from the underlying core by a subendothelial space (Figure 10). The central core of a villus contained loosely packed arachnoid cells, fibroblasts, macrophages, and bundles of collagen. The core was traversed by a network of channels (0.6-1.5 μm in width) that were in continuity with the subendothelial space



Figures 3-5. **Figure 3.** An SEM of the luminal surface of a villus showing endothelial cells (EC) displaying microvilli (MV) and cytoplasmic processes (CP). The pores (P) are seen in the crypt (CT) and at the attenuated periphery of the endothelial cells (6,800X). **Figures 4 and 5.** Scanning micrographs of the luminal surface of endothelial cells showing microvilli (MV) and pores (P) of different sizes. Note the pores with thick margins in Figure 4 (14,000X). Note the club-shaped microvilli in Figure 5 (19,000X).



Figures 6-8. **Figure 6.** An SEM of the interior surface of a villus sectioned through the middle. Note the presence of channels (CH) lined by arachnoid cells (AC) and their stout cell processes (AP) containing collagen (C) fibrils (4,500X). **Figure 7.** Light photomicrograph (LM) of a longitudinal section through an arachnoid villus showing the core containing arachnoid cells (AC) as well as channels (CH) and covered by the endothelium (END) (380X). **Figure 8.** A LM of a longitudinal section through a villus showing its endothelial cap (END) separated by a subendothelial space (SES) from the core containing arachnoid cells (AC) and channels (CH) (600X).

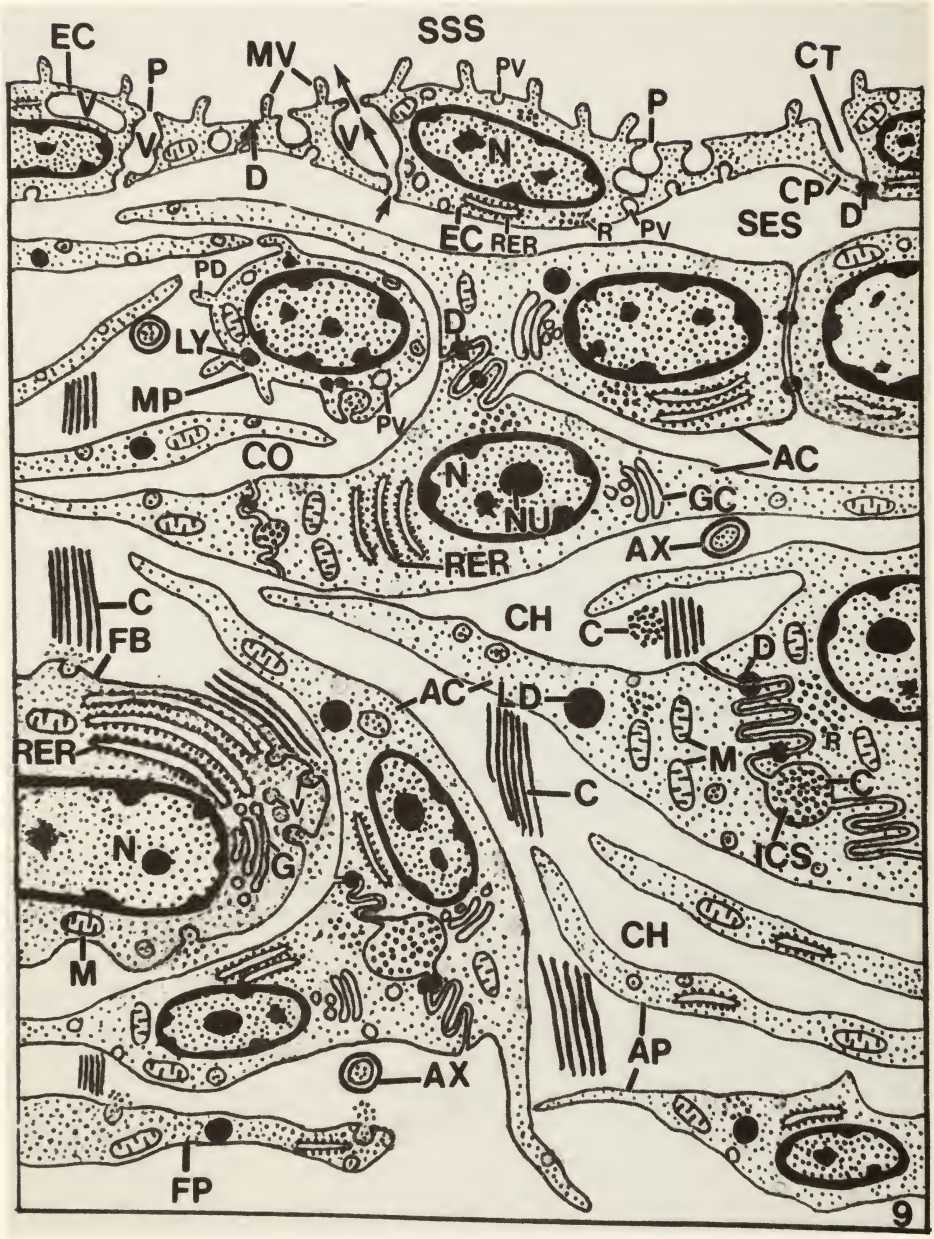
(Figures 9 and 10). In addition, the core contained fine myelinated fibers in close proximity to the arachnoid cells (Figure 10, inset). The slender interdigitating cytoplasmic processes of the endothelial cells were joined by desmosomes (Figure 11, inset). The shallow crypts occasionally seen between the endothelial cells did not open into the subendothelial sinus (Figures 11 and 12). The endothelial cells had flattened nuclei and giant vacuoles (0.4 μm in diameter), rough endoplasmic reticulum, ribosomes, and mitochondria in the cytoplasm. Numerous micropinocytotic vesicles were observed in the basal and apical cytoplasm of the endothelial cells as well as surrounding and fusing with the giant cytoplasmic vacuoles (Figures 9-13). The luminal surface of the endothelial cells displayed pores (0.27 μm in diameter) which opened into the sinus lumen. The pores communicated with the giant cytoplasmic vacuoles and their joint alignment apparently created transcellular channels (Figures 12 and 13). The pores seen in TEM appeared smaller than those seen in SEM.

The arachnoid cells were the predominant cells in the core of the villi. They were irregularly shaped and had ovoid nuclei containing clumps of chromatin located both beneath the nuclear membrane and dispersed in the nucleoplasm. The arachnoid cells contained many mitochondria, rough endoplasmic reticulum, and vesicles in their cytoplasm, but Golgi complexes were rare. The arachnoid cells had long, overlapping cytoplasmic processes that were often joined by desmosomes. The cytoplasmic processes lined the channels and often enclosed rounded intercellular spaces filled with collagen bundles (Figures 9, 14, and 15). Similar spaces in the rat arachnoid matter were described as "extracellular tunnels" by Peters, *et al.* (1976).

In addition to the arachnoid cells, fibroblasts and macrophages were also found in the core of the villi. The fibroblasts displayed vesicles fusing with the plasma membrane, apparently releasing their product by exocytosis near the cell surface for assembly into collagen fibrils in the channels and intercellular spaces (Figure 15). The macrophages were globular in shape and exhibited blunt pseudopodia at the surface. They had large, occasionally indented, nuclei, and their cytoplasm contained mitochondria, vesicles, vacuoles, and lysosomes (Figure 16). Endothelium-lined tubes and blood vessels were absent in the arachnoid villi of the squirrel monkey.

DISCUSSION

Most researchers believe that the arachnoid villi/granulations are responsible for transporting cerebrospinal fluid (CSF) from the subarachnoid space into the venous blood (Weed, 1914; d'Avilla, *et al.,* 1983; Takahashi, *et al.,* 1993). In the present study, the arachnoid villi of the squirrel monkey were shown to have a continuous covering of endothelial cells that had many pores on their luminal surface. The pores seen in SEM appeared to be larger in diameter than those seen in TEM. The apparent difference in pore size might be related to the size of the vacuoles with which they communicated. The giant vacuoles in the cytoplasm of the endothelial cells resembled those described in monkey by



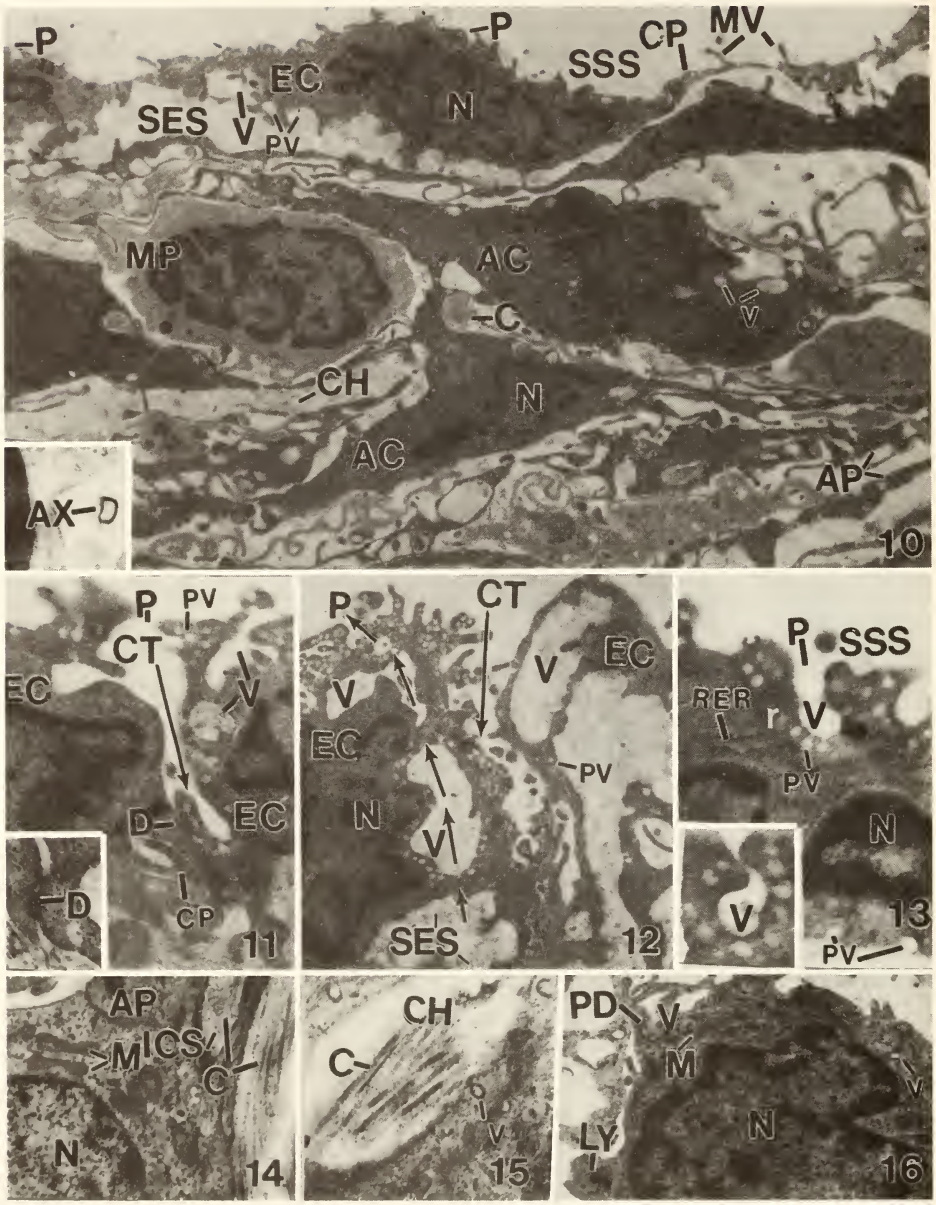
Tripathi (1973) and Takahashi, *et al.* (1993). The presence of micropinocytotic vesicles near and apparently fusing with the giant vacuoles suggests that the vesicles coalesce to form and/or increase the size of the giant vacuoles. The giant vacuoles align and coalesce with the apical pores and basal micropinocytotic vesicles, apparently creating transcellular channels through the endothelial cells. The presence of transcellular channels in the endothelial covering of the arachnoid villi strongly suggests that open intracellular communication occurs between the subarachnoid space and the venous sinus into which the villi project.

Figure 9. A pictorial summary of the basic ultrastructural features of an arachnoid villus of the squirrel monkey. A continuous layer of endothelial cells (EC) bearing microvilli (MV) covers the core of the villus. The fusiform endothelial cells have elongated nuclei (N) and slender cytoplasmic processes (CP) joined by desmosomes (D). The endothelial cells display micropinocytotic vesicles (PV) on both basal and apical surfaces and have giant vacuoles (V) in the cytoplasm which open on the apical surface through pores (P). Shallow crypts (CT) are found between the endothelial cells. The core of the villus contains arachnoid cells (AC) with long cytoplasmic processes (AP), fibroblasts (FB), macrophages (MP) with pseudopodia (PD), bundles of collagen (C), and a network of anastomosing channels (CH). The arachnoid cell processes (AP) are united by desmosomes (D) and enclose rounded intercellular spaces (ICS) containing collagen. The arrows indicate the direction of transcellular outflow of CSF from the subendothelial space (SES) into the lumen of the superior sagittal sinus (SSS). Myelinated axons (AX) are found in association with the arachnoid cells. (FP = fibroblast cell process; GC = Golgi complex; LD = lipid droplet; LY = lysosome; M = mitochondria; R = ribosomes; and RER = rough endoplasmic reticulum.)

A subendothelial space was present which resembled the structure reported in the arachnoid villi of the Macaque monkey (Shabo and Maxwell, 1968a, b). The network of anastomosing extracellar channels in the core of the villi apparently allows CSF to pass from the subarachnoid space into the subendothelial sinus. Alksne and Lovings (1972b) demonstrated in dogs that after injection of blood into the subarachnoid space, intact erythrocytes accumulated and degenerated in the channels but did not pass through the endothelium into the venous sinus. Also, in human arachnoid villi affected by subarachnoid hemorrhage, intact erythrocytes were found in the core channels (Upton and Weller, 1985; Yamashima, 1986). These observations confirm that the core channels are continuous with the subarachnoid space. Thus, the channels function as a pathway for CSF drainage.

The fact that the cytoplasmic processes of the endothelial cells, including those located at the bottom of shallow endothelial crypts, were joined by desmosomes strongly suggests that no intercellular openings exist in the endothelial covering of the arachnoid villi of the squirrel monkey. This conclusion is supported by the observation that the endothelial covering of the dog arachnoid villi remained intact but became thinner with increased intracranial pressure (Alksne and White, 1965).

The endothelium-lined tubes that have been reported in the arachnoid granulations of other mammals (Jayatilaka, 1965a, b; Hayes, *et al.,* 1971; Potts, *et al.,* 1972; Gomez and Potts, 1974; Gomez, *et al.,* 1974) were absent in the arachnoid villi of the squirrel monkey. These endothelium-lined tubes might represent long, narrow endothelial crypts which subdivide the relatively large arachnoid granulations into small arachnoid villi. Based on this view, the large arachnoid granulations merely represent clusters of small arachnoid villi which are the actual functioning units that drain CSF from the subarachnoid space into the venous system. This conclusion is supported by morphological evidence that



the endothelium-lined tubes are found only in the largest arachnoid granulations of calves (Hayes, *et al.*, 1971).

This study supports the mechanism of vacuolar transport proposed by Tripathi (1973) and Tripathi and Tripathi (1974). Based on their mechanism, the giant vacuoles formed within the endothelial cells of the villus transport CSF from their basal to their apical side into the lumen of the venous sinus. This form of CSF absorption along with its protein constituents across the endothelial cells depends upon a mechanism of bulk flow which is believed to combine features

Figures 10-16. **Figure 10.** A transmission electron micrograph (TEM) of a transverse section through the villus showing a continuous layer of fusiform endothelial cells (EC) with slender cytoplasmic processes (CP). Note the anastomosing channels (CH) lined by arachnoid cells (AC), the cytoplasmic processes (AP) of the arachnoid cells, and collagen bundles (C) in the core (8,500X). (MV = microvilli; MP = macrophage; N = nucleus; P = pores; PV = micropinocytotic vesicles; SES = subendothelial spaces; SSS = superior sagittal sinus; and V = giant vacuole.) The inset shows an axon (AX) near an arachnoid cell (18,000X). **Figure 11.** An endothelial crypt (CT) between two endothelial cells (EC) is closed off by a desmosome (D) from the subendothelial space (11,500X). (P = pore; PV = micropinocytotic vesicles; and V = vacuoles.) The inset shows a desmosome (D) uniting the endothelial cell processes (37,000X). **Figure 12.** A TEM displaying endothelial cells (EC) containing giant vacuoles (V) and numerous micropinocytotic vesicles (PV). (CT = endothelial crypt; N = nucleus; P = pore; SES = subendothelial space.) The arrows indicate outflow of CSF from the subendothelial space (SES) into the sinus lumen through the pores (9,000X). **Figure 13.** A portion of an endothelial cell showing micropinocytotic vesicles (PV) in the basal and apical cytoplasm. The vesicles are seen surrounding and some opening into giant vacuoles (V) which, in turn, open through pores (P) into the lumen of the superior sagittal sinus (SSS). Note the ribosomes (r) and the rough endoplasmic reticulum (RER) in the cytoplasm (35,000X). (N = nucleus.) The inset shows a giant vacuole (V) opening into the sinus lumen through a narrow pore (51,000X). **Figure 14.** A portion of an arachnoid cell displaying cytoplasmic processes (AP) that enclose an intercellular space (ICS) containing collagen fibrils (C). (M = mitochondria and N = nucleus.) (10,000X). **Figure 15.** A portion of a fibroblast showing a smooth vesicle (v) in contact with the plasma membrane and collagen (C) fibrils in the channel (CH). (22,000X.) **Figure 16.** Part of an active macrophage in the villus core displaying pseudopodia (PD), mitochondria (M), lysosomes (LY), and nucleus (N). (8,300X).

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of both the "closed" and "open" systems (Tripathi and Tripathi, 1974; Kendel, *et al.,* 1991; Fishman, 1992). That bulk flow is a pressure-dependent process is supported by the observation that, with CSF hypotension, the giant vacuoles involved in this process are not formed in the endothelial covering of monkey arachnoid granulations, which also appear smaller in size (Takahashi, *et al.,* 1993). The presence of numerous pinocytotic vesicles at the basal and apical surfaces of the endothelial cells indicated that these cells were also engaged in the passive diffusion of smaller molecules (McComb, 1983).

The central core of the arachnoid villi contained three cell types: arachnoid cells, fibroblasts, and macrophages. The predominant arachnoid cells had long, overlapping cytoplasmic processes often joined by desmosomes which apparently maintained the longitudinal orientation of the core channels to facilitate CSF flow. The fibroblasts displayed ultrastructural features resembling those reported in the arachnoid villi of calves (Hayes, *et al.,* 1971) and humans (Kida, *et al.,* 1988). The fibroblasts apparently synthesize the collagen found in the intercellular spaces and core channels.

The presence of macrophages in the villi indicates that they might be involved in removing and destroying particulate material, such as viruses, bacteria, and dead cells, from the CSF. Furthermore, the macrophages might engulf calcarious material which has been reported to develop in the arachnoid matter with advancing age (Millen and Woolam, 1962). Macrophages have been reported in the subendothelial space of the arachnoid villi of the Macaque monkey (Shabo and Maxwell, 1968b), and their presence has been suggested in human arachnoid granulations (Upton and Weller, 1985).

The present study confirmed the presence of fine, myelinated axons in the arachnoid villi of the squirrel monkey. Innervention of the arachnoid granulations in sheep and monkey had been reported earlier by Jayatilaka (1965b) and von Düring and Andres (1991), respectively. The absence of blood vessels suggests that the nerve fibers reported here are sensory in function.

ACKNOWLEDGMENTS

The author wishes to thank Dr. S.L. Manocha, Yerkes Primate Research Center, Atlanta, Georgia, for providing and anesthetizing the squirrel monkey and for the use of his laboratory facilities. The author also thanks his colleagues Drs. Lee Engstrom and Stuart Walker for their critical reading of the manuscript. Thanks are due to Dr. Larry Ganion for the use of RCA-3C electron microscope. The author is also grateful to Mrs. Beth Verhoestra for technical assistance and to Mrs. Lisa Garrison for her skillful typing of the manuscript. The author is further grateful to the library staff of Ball Memorial Hospital, Muncie, Indiana, for a medline literature search and for obtaining interlibrary loan articles.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 175-189

GENETIC AND ENVIRONMENTAL EFFECTS OF SIDESTREAM SMOKE ON PUP SURVIVORSHIP OF THREE INBRED STRAINS OF MICE

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ABSTRACT: Crosses and backcrosses of three inbred mouse strains, BALB/c, C57BL, and C3H, were made to study the effects of pup genetic composition and sidestream smoke exposure on *in utero* pup survivorship. Pups that varied in genetic composition were produced by making the appropriate crosses and backcrosses of these inbred strains. A Model I ANOVA showed that pup survivorship for all three strain crosses and backcrosses was significantly affected both by the genetic composition of the pups and by sidestream smoke exposure ($P < 0.001$). Analysis of variance also indicated that significant interaction between genetic composition and smoke exposure occurred only in the BALB/c x C57BL crosses and backcrosses. Variation in the survivorship for pups of different genetic composition was identified using Tukey's multiple comparison test. The only comparison of pups differing in genetic composition that consistently resulted in significant differences in survivorship was between 0% and 75% genetic composition. Several significant differences in mean percent survivorship between the control and smoke-exposed pups were also found. For example, control pups with 75% of their genes derived from strain C3H (produced by C3H x BALB/c crosses and backcrosses) had a mean percent survivorship that was significantly greater than that of sidestream smoke-exposed pups (63.8% for the controls in contrast to 46.0% for the experimentals; $P < 0.001$). Our findings suggest that both sidestream smoke and genetic composition significantly affect pup survivorship.

KEYWORDS: Genetic composition, passive smoking, sidestream smoke, survivorship, tobacco smoke exposure.

INTRODUCTION

Tobacco smoking is a major cause of morbidity and mortality. Smoking is responsible for an estimated 434,000 premature deaths each year in the United States (Center for Disease Control, 1988) and up to 3 million each year worldwide (Schwarz and Schmeiser-Rieder, 1996). Many of the deleterious substances found in mainstream smoke are also present in sidestream smoke. These substances include ammonia, carbon monoxide, aromatic amines, nitrosamines, and polycyclic hydrocarbons (U.S. Department of Health and Human Services, 1986). The concentrations of tar, nicotine, carbon monoxide, and carbon dioxide as well as benzo(a)pyrene, a known carcinogen, are 2 to 12 times higher in sidestream smoke than in mainstream smoke (Adams, *et al.*, 1987; Eatough, *et al.*, 1990; Guerin, *et al.*, 1992; Morris, 1995). Aqueous extracts from cigarette tar can bind to and nick DNA (Stone, *et al.*, 1995).

Because of these disturbing facts, concern is growing that involuntary exposure to sidestream smoke (passive smoking) produces risks for many of the same health hazards as does active smoking. Sidestream smoke exposure decreases pulmonary function (Sun, *et al.*, 1995), is associated with asthma and other respiratory illness in the children of active smokers (Chilmonczyk, *et al.*, 1993; Cunningham, *et al.*, 1996; Halken, *et al.*, 1995), and increases adult non-neoplastic lung disease (Tredaniel, *et al.*, 1994). Passive smoking also increases the risk of lung cancer (Brownson, *et al.*, 1992; Liu, *et al.*, 1993; Morris, 1995; Stockwell, *et al.*, 1992) and heart disease (Glantz and Parmley, 1995; Kawachi, *et al.*, 1997; Steenland, 1992). Overall estimates of the number of annual passive smoking deaths range from 39,000 to 53,000 (Wells, 1988).

Ample evidence exists to show that maternal smoking during pregnancy constitutes a hazard to the fetus. Smoking retards fetal growth (Nash and Persaud, 1988; Wilcox, 1993), producing low birth weight offspring (Chen, *et al.*, 1989; Lazzaroni, 1990) and increases the risks of spontaneous perinatal death and neonatal morbidity (Abel, 1980; U.S. Department of Health, Education, and Welfare, 1979). Whether or not pregnant women exposed to sidestream smoke expose the fetus to such risks is less clear. Recent research seems to indicate that fetal growth is reduced by exposure to sidestream smoke in pregnant women (Chen, *et al.*, 1989; Chen and Petitti, 1995; Fortier, *et al.*, 1994). Passive smoking during pregnancy is also a risk factor for persistent pulmonary hypertension in the newborn (Bearer, *et al.*, 1997). A variety of studies on pregnant animals exposed to sidestream smoke have demonstrated some of the same deleterious offspring effects experienced by the fetus of an active smoker (Abel, 1980; Bassi, *et al.*, 1984; Essenberg, *et al.*, 1940; Leichter, 1993; Mays, 1986; Mays, *et al.*, 1988, 1993; Mays, *et al.*, 1994; Reznik and Marquard, 1980; Vahakangas, *et al.*, 1982).

Although recent research has substantiated individual variability among both humans and other animals to the effects of tobacco smoke and nicotine (Overstreet, 1995; Parrot, 1994; Perkins, 1995; Shiffman, 1989; Swan, *et al.*, 1993), concern is growing that the potential effect of passive smoking among pregnant women is a serious issue (Chen and Petitti, 1995). This concern exists because of the prevalence of passive smoking among women of reproductive age and the fact that some substances in tobacco smoke can cross the placental barrier (Smith and Austen, 1982).

Any study of the effects of an environmental factor on phenotypic variation (V_P) has the inherent problem of separating the genetic variation (V_G) from that of the treatment (V_E). In this investigation, we used three inbred strains of mice and their hybrids to evaluate whether or not maternal exposure to sidestream smoke affects pup survival and to see if genetically based differences in survivorship exist.

MATERIALS AND METHODS

Three sets of crosses involving three inbred mouse strains, BALB/cAnHsd, C57BL/6NHsd, and C3H/HeNHsd, were performed following Hayes (1987).

Briefly, the three strains were crossed in all possible combinations, and the pregnant females were exposed to sidestream smoke. For any one set of crosses, the pups either contained 50% of their genes from each parental strain (i.e., hybrid pups) or 25% of their genes from one of the parental strains and 75% of their genes from another strain. We also collected data on purebred pups for comparison. Throughout the paper the crosses will simply be referred to as either BALB/c x C57BL, C57BL x C3H, or C3H x BALB/c with symbol designates as: B = BALB/c, C = C57BL, 3 = C3H, and H = hybrid. The first letter of a cross represents the dam. For example, BxH(BxC) is the cross of a BALB/c dam and a hybrid (H) sire whose mother was BALB/c and whose father was C57BL. Seventy-five percent of the genes in the pups from such a cross will be BALB/c in origin.

Virgin female mice were mated overnight with appropriate males. The day a copulation plug was visually observed was designated as gestation day 1. Pregnant mice were maintained in separate cages under controlled environmental conditions for room temperature (20-25° C), humidity (55-60%), and photoperiod (12 hr light, 12 hr dark). They were fed Lab Blox pellets (Wayne Feed Division, Continental Grain Co.) and water *ad libitum* between trials.

Experimental mice (pregnant dams) were placed in a Plexiglas smoking chamber (25.5 cm x 30.5 cm x 21.0 cm) containing 6 air holes, each 6.5 mm in diameter, on two opposite sides and exposed each day of gestation (21 days) to sidestream smoke from one commercial brand filter-tip cigarette. The tar and nicotine content of the cigarettes used was 15.1 mg and 0.13 mg, respectively. These values are approximately in the mid-range of the tar and nicotine content of 25 popular commercial cigarette brands (ranging from a low of 1.0 mg tar/0.13 mg nicotine to a high of 29.8 mg tar/2.03 mg nicotine (Federal Trade Commission, 1997)). The duration of each smoking session was 40 minutes. At the end of each treatment period, the dams were returned to their respective cages. Treatment was terminated at parturition, and the dams were allowed to nurture their pups until they were weaned at day 21. On postnatal day 1 (within 16 hr of birth), each experimental pup was sexed and weighed. Percent survivorship values were calculated for each litter and were based on the number of pups alive at day 21 relative to the number of pups born. Control mice were treated in the same manner as the experimentals except that the smoking chamber contained an unlit cigarette.

Statistical analyses were made using the Minitab program. Percent survivorship values were arcsine transformed and analyzed separately for each set of crosses using a Model I ANOVA. The outcome of the ANOVA served as the basis for Tukey's multiple comparison test (Zar, 1984) to test for differences in the percent survivorship among control and smoke-exposed pups with different percent genetic composition values. Differences in percent survivorship between the control and experimental pups at different percent genetic composition values were also determined by this method. Experiment-wise error rates were set at $\alpha = 0.05$.

Table 1. Mean pup survivorship based on % BALB/c genetic composition produced by BALB/c (B) x C57BL (C) crosses and backcrosses.

Treatment	% BALB/c Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON[3]	0% (100% C57BL)	$\sum$ = **70**	CxC	$\bar{x}$ = **56.445**	2.679
	25% (75% C57BL)	20	CxH(BxC)	74.740	
		23	CxH(CxB)	80.594	
		36	H(BxC)xC	84.130	
		17	H(CxB)xC	84.618	
		$\sum$ = **96**		$\bar{x}$ = **81.021**	2.288
	50% (50% C57BL)	32	BxC	72.725	
		38	CxB	79.364	
		43	H(BxC)	80.238	
		17	H(CxB)	79.508	
		$\sum$ = **130**		$\bar{x}$ = **77.959**	1.966
	75% (25% C57BL)	34	BxH(BxC)	77.849	
		29	BxH(CxB)	78.670	
		14	H(BxC)xB	84.956	
		16	H(CxB)xB	80.542	
		$\sum$ = **93**		$\bar{x}$ = **80.504**	2.325
	100% (0% C57BL)	$\sum$ = **40**	BxB	$\bar{x}$ = **76.400**	3.545
EXP[3]	0% (100% C57BL)	$\sum$ = **34**	CxC	$\bar{x}$ = **40.120**	4.314
	25% (75% C57BL)	16	CxH(BxC)	66.607	
		33	CxH(CxB)	50.367	
		16	H(BxC)xC	81.461	
		14	H(CxB)xC	75.483	
		$\sum$ = **79**		$\bar{x}$ = **68.480**	2.522
	50% (50% C57BL)	67	BxC	68.547	
		30	CxB	73.662	
		85	H(BxC)	71.006	
		13	H(CxB)	76.148	
		$\sum$ = **195**		$\bar{x}$ = **72.341**	1.605
	75% (25% C57BL)	12	BxH(BxC)	80.239	
		16	BxH(CxB)	71.659	
		29	H(BxC)xB	81.707	
		15	H(CxB)xB	79.529	
		$\sum$ = **72**		$\bar{x}$ = **78.284**	2.642
	100%	$\sum$ = **26**	BxB	$\bar{x}$ = **67.781**	4.396

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON = Control; EXP = Experimental.

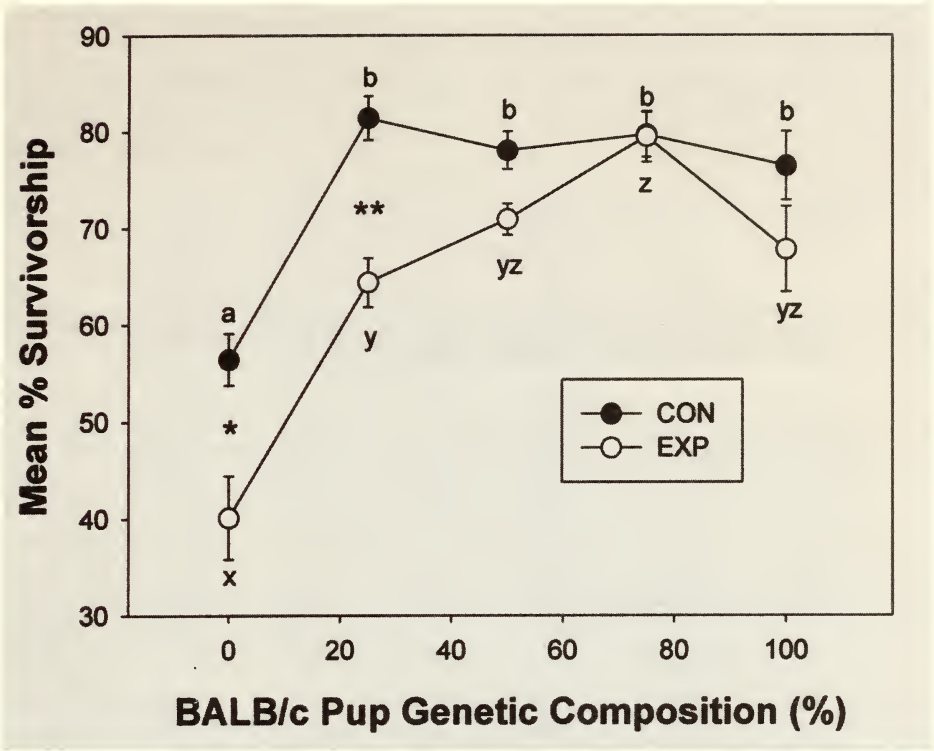

Figure 1. A comparison of the arcsine tranformed control and experimental pup mean percent survivorship values based on the percent BALB/c genetic composition produce by BALB/c x C57BL crosses and backcrosses. The *P* values were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$; ** = significant at $P < 0.001$). Means with no letters in common are significantly different at $P < 0.001$.

RESULTS

Arcsine transformed percent survivorship values for the BALB/c x C57BL, C57BL x C3H, and C3H x BALB/c crosses are presented in Tables 1-3, respectively. Analysis of variance of the BALB/c x C57BL crosses indicated that both the percent genetic composition of the pups and sidestream smoke exposure significantly affected pup percent survivorship ($P < 0.001$; Table 1). Analysis of variance also indicated significant interaction between the two factors (Table 4a). Tukey's multiple comparison tests were performed to distinguish differences in the percent survivorship values both among and between control and smoke-exposed pups with different percent genetic composition values. The percent survivorship of control pups with 0% BALB/c (100% C57BL) genes differed significantly ($P < 0.001$) from that of pups with 25%, 50%, 75%, and 100% BALB/c genes (Table 5). Experimental pups displayed similar percent survivorship differences, but in addition, the percent survivorship of pups with 25% BALB/c genetic composition differed significantly ($P < 0.001$) from that of pups

Table 2. Mean pup survivorship based on % C57BL genetic composition produced by C57BL x C3H crosses and backcrosses.

Treatment	% C57BL Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON + EXP[3]	0% (100% C3H)	32 (Con)	3x3	64.394	
		34 (Exp)	3x3	61.865	
		Σ = 66		**$\bar{x}$ = 63.130**	3.038
	25% (75% C3H)	20 (Con)	3xH(Cx3)	61.272	
		19 (Con)	3xH(3xC)	67.740	
		21 (Con)	H(Cx3)x3	87.367	
		20 (Con)	H(3xC)x3	88.105	
		80 (Con)		**76.121**	
		20 (Exp)	3xH(Cx3)	48.407	
		21 (Exp)	3xH(3xC)	53.411	
		21 (Exp)	H(Cx3)x3	78.423	
		21 (Exp)	H(3xC)x3	86.311	
		83 (Exp)		**66.638**	
		Σ = 163		**$\bar{x}$ = 71.380**	1.933
	50% (50% C3H)	16 (Con)	Cx3	62.455	
		18 (Con)	3xC	76.512	
		12 (Con)	H(Cx3)	77.943	
		18 (Con)	H(3xC)	85.000	
		64 (Con)		**75.478**	
		29 (Exp)	Cx3	55.970	
		21 (Exp)	3xC	76.513	
		21 (Exp)	H(Cx3)	75.605	
		22 (ExpP	H(3xC)	78.302	
		93 (Exp)		**71.598**	
		Σ = 157		**$\bar{x}$ = 73.538**	2.003
	75% (25% C3H)	16 (Con)	CxH(Cx3)	78.821	
		21 (Con)	CxH(3xC)	76.641	
		21 (Con)	H(Cx3)xC	85.458	
		21 (Con)	H(3xC)xC	83.425	
		79 (Con)		**81.092**	
		20 (Exp)	CxH(Cx3)	60.633	
		14 (Exp)	CxH(3xC)	69.255	
		17 (Exp)	H(Cx3)xC	79.330	
		14 (Exp)	H(3xC)xC	87.068	
		65 (Exp)		**74.073**	
		Σ = 144		**$\bar{x}$ = 48.283**	2.066
	100% (0% C3H)	70 (Con)	CxC	54.445	
		34 (Exp)	CxC	40.121	
		Σ = 104		**$\bar{x}$ = 48.283**	2.794

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON + EXP = Control + Experimental.

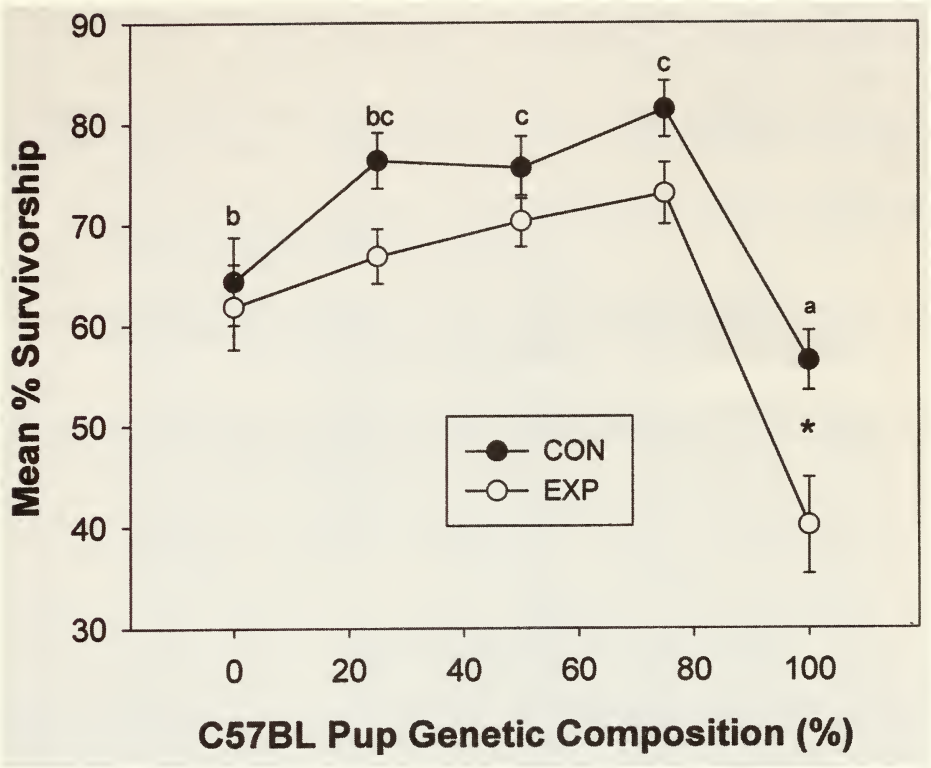

Figure 2. A comparison of the arcsine transformed control and experimental pup mean percent survivorship values based on the percent C57BL genetic composition produced by C57BL x C3H crosses and backcrosses. The values of P were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$). Means with no letters in common are significantly different at $P < 0.05$.

with 75% BALB/c genetic composition. Control pups with 0% and 25% BALB/c genetic composition had significantly higher percent survivorship values than did the corresponding experimental pups. At 0% genetic composition, control pup percent survivorship was 56.5%, and experimental pup percent survivorship was 40.1% ($P < 0.05$); at 25% genetic composition, control pup percent survivorship was 81.0%, and experimental pup percent survivorship was 68.5% ($P < 0.001$; Figure 1).

Analysis of variance of the C57BL x C3H crosses showed that both the percent genetic composition of the pups and the effects of sidestream smoke exposure significantly affected pup percent survivorship ($P < 0.001$; Table 2). No interaction between the percent C57BL genetic composition of the pups and smoke exposure was indicated (Table 4b). Therefore, Tukey's multiple comparison test was performed on the pooled percent survivorship values of the control and experimental pups to determine differences in percent survivorship between groups with different genetic compositions. The percent survivorship

Table 3. Mean pup survivorship based on % C3H genetic composition produced by C3H x BALB/c crosses and backcrosses.

Treatment	% C3H Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON + EXP[3]	0% (100% BALB/c)	40 (Con)	BxB	76.400	
		26 (Exp)	BxB	67.781	
		Σ = 66		**$\bar{x}$ = 72.091**	3.000
	25 % (75% BALB/c)	24 (Con)	BxH(Bx3)	77.172	
		31 (Con)	BxH(3xB)	70.772	
		32 (Con)	H(Bx3)xB	83.969	
		32 (Con)	H(3xB)xB	82.806	
		119 (Con)		**78.681**	
		30 (Exp)	BxH(Bx3)	61.570	
		22 (Exp)	BxH(3xB)	61.227	
		26 (Exp)	H(Bx3)xB	74.389	
		20 (Exp)	H(3xB)xB	78.000	
		98 (Exp)		**68.797**	
		Σ = 217		**$\bar{x}$ = 73.739**	1.624
	50% (50% BALB/c)	20(Con)	Bx3	75.761	
		13 (Con)	3xB	74.113	
		30 (Con)	H(Bx3)	75.711	
		32 (Con)	H(3xB)	71.256	
		95 (Con)		**74.210**	
		25 (Exp)	Bx3	74.191	
		36 (Exp)	3xB	62.475	
		24 (Exp)	H(Bx3)	74.014	
		28 (ExpP	H(3xB)	65.395	
		113 (Exp)		**69.041**	
		Σ = 208		**$\bar{x}$ = 71.626**	1.648
	75% (25% BALB/c)	31 (Con)	3xH(Bx3)	59.308	
		32 (Con)	3xH(3xB)	44.143	
		26 (Con)	H(Bx3)x3	79.594	
		30 (Con)	H(3xB)x3	72.328	
		119 (Con)		**63.843**	
		21 (Exp)	3xH(Bx3)	19.945	
		24 (Exp)	3xH(3xB)	23.065	
		23 (Exp)	H(Bx3)x3	72.773	
		25 (Exp)	H(3xB)x3	68.247	
		93 (Exp)		**46.007**	
		Σ = 212		**$\bar{x}$ = 54.925**	1.648
	100% (0% BALB/c)	32 (Con)	3x3	64.394	
		34 (Exp)	3x3	61.865	
		Σ = 104		**$\bar{x}$ = 63.130**	2.933

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON + EXP = Control + Experimental

Table 4. Summary of results generated by a Model I ANOVA of arcsine transformed % survivorship values attributed to the percent genetic composition of three inbred mouse strains: **A**) %BALB/c genetic composition (BALB/c x C57BL crosses and backcrosses); **B**) % C57BL genetic composition (C57BL x C3H crosses and backcrosses); and **C**) % C3H genetic composition (C3H x BALB/c crosses and backcrosses). Significance at $P < 0.05$.

	ANOVA: Linear Model I	*F*	*P*
A	BALB/c Genetic Comp (%)	27.36	0.000
	BALB/c Smoke Effects	27.56	0.000
	Interaction (Genetic Comp and Smoke Effects)	3.60	0.006
B	C57BL Genetic Comp (%)	20.09	0.000
	C57BL Smoke Effects	15.22	0.000
	Interaction (Genetic Comp and Smoke Effects)	0.900	.462
C	C3H Genetic Comp (%)	20.14	0.000
	C3H Smoke Effects	18.63	0.000
	Interaction (Genetic Comp and Smoke Effects)	1.74	0.139

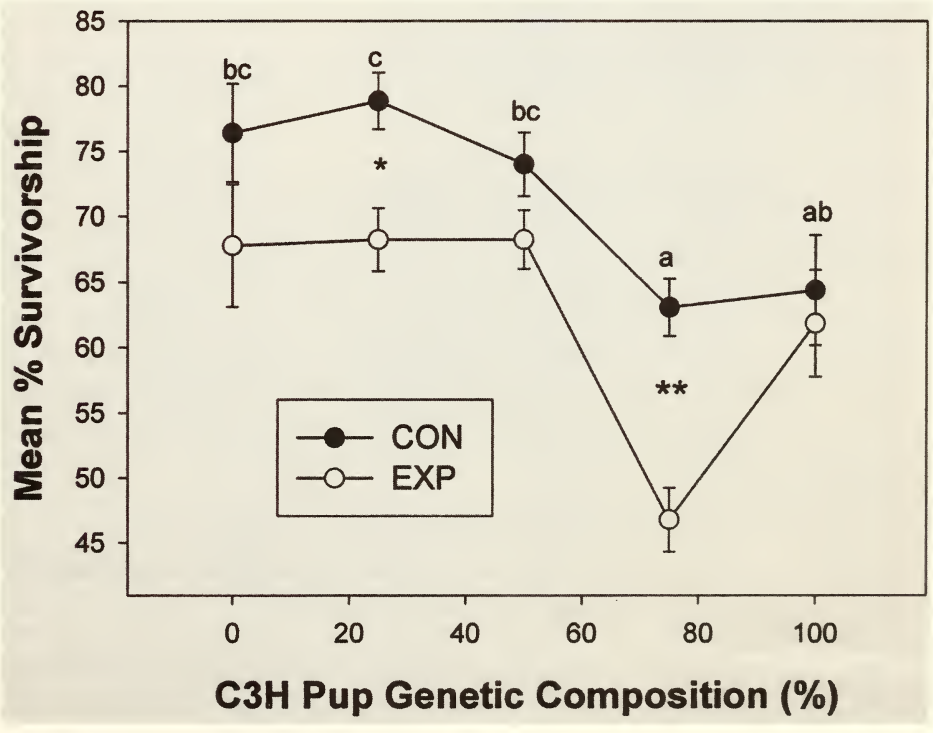

Figure 3. A comparison of the arcsine transformed control and experimental pup mean percent survivorship values based on the percent C3H genetic compostion produced by C3H x BALB/c crosses and backcrosses.The *P* values were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$; ** = significant at $P < 0.001$). Means with no letters in common are significantly different at $P < 0.05$.

Table 5. Tukey's multiple comparison tests of the differences between pup survivorship at different percent BALB/c genetic composition values produced by BALB/c x C57BL crosses.

Treatment	Groups Compared (% BALB/c Genetic Composition)	q
Controls	0 vs 25	10.017**
	0 vs 50	9.186**
	0 vs 75	9.244**
	0 vs 100	6.300**
	25 vs 50	1.598
	25 vs 75	0.774
	25 vs 100	1.689
	50 vs 75	0.759
	50 vs 100	0.572
	75 vs 100	1.093
Experimentals	0 vs 25	7.668**
	0 vs 50	9.466**
	0 vs 75	10.994**
	0 vs 100	6.350**
	25 vs 50	3.078
	25 vs 75	6.516**
	25 vs 100	0.905
	50 vs 75	3.904
	50 vs 100	0.946
	75 vs 100	3.209
Controls vs Experimentals	0 vs 0	4.549*
	25 vs 25	7.060**
	50 vs 50	3.976
	75 vs 75	0.080
	100 vs 100	2.158

* Significant at $P = 0.05$
** Significant at $P = 0.001$

of the pups with 0% C57BL genetic composition differed significantly from that of pups with 50% ($P < 0.05$), 75% ($P < 0.005$), and 100% ($P < 0.005$) C57BL genetic composition. The percent survivorship of pups with 25%, 50%, and 75% C57BL genetic composition differed significantly from that of pups with 100% C57BL genetic composition ($P < 0.001$; Table 6). Control pups with 100% C57BL genetic composition had a significantly higher percent survivorship than corresponding smoke-exposed pups (54.5% in contrast to 40.1%; $P < 0.05$; Figure 2).

Analysis of variance of the C3H x BALB/c crosses showed that both the percent C3H genetic composition of the pups and sidestream smoke exposure sig-

Table 6. Tukey's multiple comparison tests of the differences between pup survivorship at different percent C57BL genetic composition values produced by C57BL x C3H crosses.

Treatment	Groups Compared (% C57BL/Genetic Composition)	q
Controls + Experimentals	0 vs 25	3.355
	0 vs 50	3.901*
	0 vs 75	5.445**
	0 vs 100	5.384**
	25 vs 50	0.710
	25 vs 75	2.847
	25 vs 100	10.573***
	50 vs 75	2.118
	50 vs 100	11.061***
	75 vs 100	12.775***
Controls vs Experimentals	0 vs 0	0.597
	25 vs 25	3.483
	50 vs 50	1.949
	75 vs 75	3.209
	100 vs 100	4.493*

* Significant at $P = 0.05$
** Significant at $P = 0.005$
*** Significant at $P = 0.001$

nificantly affected percent survivorship ($P < 0.001$; Table 3). No interaction between the percent C3H genetic composition and smoke exposure was indicated (Table 4c). Due to the lack of interaction, Tukey's multiple comparison test was run on the pooled percent survivorship values of the control and experimental pups to determine differences in percent survivorship between groups with different genetic compositions. The percent survivorship of pups with 0% C3H genetic composition differed significantly from that of pups with 75% C3H genetic composition ($P < 0.001$). The percent survivorship of pups with 25% C3H genetic composition differed significantly from that of pups with C3H genetic composition values of 75% ($P < 0.001$) and 100% ($P < 0.025$), and the percent survivorship of pups with 50% C3H genetic composition differed significantly from that of pups with 75% C3H genetic composition ($P < 0.001$). Control pups with 25% and 75% C3H genetic composition had significantly higher percent survivorship values than did the corresponding experimental pups. At 25% C3H genetic composition, control pup percent survivorship was 78.7%, and experimental pup percent survivorship was 68.8% ($P < 0.05$). At 75% C3H genetic composition, control pup percent survivorship was 63.8%, and experimental pup percent survivorship was 46.0% ($P < 0.001$; Figure 3).

Table 7. Tukey's multiple comparison tests of the differences between pup survivorship at different percent C3H genetic composition values produced by C3H x BALB/c crosses.

Treatment	Groups Compared (% C3H Genetic Composition)	*q*
Controls + Experimentals	0 vs 25	0.619
	0 vs 50	0.40
	0 vs 75	7.241 ***
	0 vs 100	3.072
	25 vs 50	1.482
	25 vs 75	11.466 ***
	25 vs 100	4.420 **
	50 vs 75	9.870 ***
	50 vs 100	3.376
	75 vs 100	3.469
Controls vs Experimentals	0 vs 0	2.032
	25 vs 25	4.629 *
	50 vs 50	2.490
	75 vs 75	7.056 ***
	100 vs 100	0.613

* Significant at $P = 0.05$
** Significant at $P = 0.025$
*** Significant at $P = 0.001$

In order to determine if the sex of the pups had an effect on percent survivorship, we calculated the pup sex ratios for all control and experimental crosses and backcrosses for day 1 and day 21. In each case, the sex ratio was approximately 1.0. Therefore, the sex of the pups did not appear to have a significant effect on percent survivorship.

DISCUSSION

In this investigation, we examined the effects of sidestream smoke exposure *in utero* and pup genetic composition on mean percent survivorship of mouse pups produced by BALB/c (B) x C57BL (C), C57BL (C) x C3H (3), and C3H (3) x BALB/c (B) crosses (Tables 1-3). The results showed that pup percent survivorship values for all three crosses were significantly affected both by the genetic composition of the pups and by sidestream smoke exposure. In addition, our results indicated that significant interaction between the two factors (percent genetic composition and smoke exposure) occurred only with the BALB/c x C57BL crosses.

Variability in the percent survivorship of pups with different genetic composition was also apparent. For example, the percent survivorship of both the control (56.5%) and experimental pups (40.1%) with 0% BALB/c (100% C57BL) genetic composition differed significantly from the percent survivorship of the control (81.0%) and experimental pups (68.5%) with 25% BALB/c (75% C57BL) genetic composition (Table 5). Neither the C57BL x C3H nor the C3H x BALB/c crosses produced significant percent survivorship differences between 0% and 25% genetic composition. The only difference in the genetic composition of BALB/c, C57BL, and C3H pups that resulted in consistently significant differences in percent survivorship was between 0% and 75% genetic composition.

Several significant differences in percent survivorship between the control and smoke-exposed pups were also noted. In the BALB/c x C57BL crosses, both 0% BALB/c genetic composition (56.5% controls/40.1% experimentals) and 25% BALB/c genetic composition (81.0% controls/68.5% experimentals) produced significant differences in pup percent survivorship. The percent survivorship of the control pups produced by C57BL x C3H crosses that had 100% C57BL genetic composition was significantly different from the percent survivorship of smoke-exposed pups (54.5% controls/40.1% experimentals). Control pups with 75% C3H genetic composition produced by C3H x BALB/c crosses had a percent survivorship that differed significantly from that of smoke-exposed pups (63.8% controls/ 46.0% experimentals).

Overall, our results suggest that, while sidestream smoke clearly affects pup survival, genetic factors contribute to the variation in susceptibility of pups to the effects of maternal exposure to sidestream smoke.

ACKNOWLEDGMENTS

The authors wish to thank Julko Fullop, Arpitha Marri, Durga Pai, Richard Ryland, Gregory Seitz, Scott Thornburg, and Mindy Wheeler for their contributions to this investigation. Thanks are also extended to Dr. Wade Hazel for his insightful comments on the manuscript and to Dr. James Benedix for his help with the statistical analysis. This research was supported in part by grants from the Howard Hughes Research Foundation, the DePauw University Faculty Development Fund, and the DePauw University Science Research Fellows Research Fund.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 191-200

THE DIFFERENCE BETWEEN MALATE SYNTHASE SPECIFIC ACTIVITY OF LIGHT AND DARK SPORED AGARICS IS NOT DUE TO PHENOLIC CONTAMINATION

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ABSTRACT: The significant difference in specific activity of malate synthase between light and dark spored agarics is not due to phenolic contamination associated with dark spored wall pigments. First, the specific activity of malate synthase was determined for individual species. Then, the specific activity of malate synthase was measured for combined homogenates (i.e., a dark spored species combined with a light spored species). If no interfering phenolic compounds were present, the observed specific activity of malate synthase for the combined homogenate should equal the expected specific activity of malate synthase (i.e., the sum of the individual specific activity of malate synthase for the two species combined divided by two). Results of such assays found no significant difference between observed and expected specific activity of malate synthase for combined homogenates. The taxonomic implications of these findings are discussed.

KEY WORDS: Enzyme assay, gilled mushrooms, glyoxylate cycle, homobasidiomycetes, malate synthase.

INTRODUCTION

While conducting a survey to determine the ubiquity of the glyoxylate cycle in basidiospores of homobasidiomycetes, Ruch, *et al.* (1991) and Ruch and

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Table 1. Specimens used in this study and their Ball State University Herbarium Mycology numbers. Specimens are arranged by family (Miller, 1979).

Species	BSU Herbarium Number
Lepiotaceae (Spores primarily white)	
Lepiota americana Peck	18
Tricholomataceae (Spores primarily white)	
Armillariella mellea (Fr.) Karst	100
Armillariella tabescens (Scop. ex Fr.) Singer	1
Flammulina velutipes (Fr.) Singer	170
Laccaria ochropurpurea (Berk.) Peck	67, 68
Lactarius psammicola var. *glaba* Smith	79, 80
Lentinellus ursinus (Fr.) Kuehn.	129
Leucoagaricus naucinus (Fr.) Singer	87
Mycena leaiana (Beck.) Sacc.	21
Oudemansiella radicata (Fr.) Singer	25, 66
Phyllotopsis nidulans (Pers. ex Fr.) Singer	128
Tricholomopsis platyphylla (Fr.) Singer	19
Pluteaceae (Spores pink)	
Pluteus granularis Peck	154
Cortinariaceae (Spores shades of brown to bright rusty-orange)	
Cortinarius sp.	151
Gymnopilus liquiritiae (Pers.) Karst.	47
Agaricaceae (Spores chocolate-brown to blackish-brown in age)	
Agaricus campestris Fr.	50, 89
Bolbitiaceae (Spores rusty or earthy brown)	
Agrocybe dura (Fr.) Singer	82
Coprinaceae (Spores deep brown to black)	
Coprinus insignus Peck	94, 102
Coprinus micaceus (Bull. ex Fr.) Fries	9, 12
Panaeolus foenisecii (Fr.) Kuehn.	17
Psathyrella velutina (Fr.) Singer	62
Strophariaceae (Spores purple-brown to purple-black or black)	
Stropharia ambigua (Pk.) Zeller	15

Samuel (1992) reported some intriguing results concerning light spored and dark spored agarics. The dark spored agarics (Families Agaricaceae, Bolbitiaceae, and Coprinaceae) possessed much lower specific activity of malate synthase then did the white (Families Amanitaceae, Lepiotaceae, Hygrophoraceae, Russulaceae, and Tricholomataceae), pink (Families Volvariaceae and Rhodophyllaceae), and light brown (Family Cortinariaceae) spored agarics. By using the Mann-Whitney test (Zar, 1974), a nonparametric analog to the two-sample *t*-test, the dark spored agarics demonstrated significantly lower malate synthase specific activity than the lighter spored species (Mann-Whitney Test: $U = 280$, $P < 0.001$).

A possible explanation for the observed difference in malate synthase activity between light and dark spored agarics is the presence of contaminating phenolic compounds associated with the dark pigments of the wall. Since phenolic compounds can function as enzyme inhibitors, their presence might account for the low specific activity demonstrated in the dark spored species. Ruch, *et al.* (1991) and Ruch and Samuel (1992) did not test for the presence of such contaminants in homogenates of dark spored mushrooms. This study was undertaken to determine if the difference in malate synthase activity between light and dark spored agarics is due to phenolic interference or implies a taxonomic phenomenon.

MATERIAL AND METHODS

The mushrooms used in this study are listed in Table 1 along with their Ball State University Herbarium mycology numbers (BSUHM). Homogenates were prepared as previously described (Cooper and Beevers, 1969; Ruch, *et al.*, 1991). Spores were homogenized in a Braun MKS cell homogenizer for a total of 60 sec at 4° C. The homogenate was centrifuged for 10 min at 500x g at 4° C. After removing any lipids that had collected at the surface, the supernatant was used for enzyme assays. Malate synthase activity was assayed by measuring the formation of a yellow complex between DTNB (5,5-dithiobisnitrobenzoic acid) and the sulfhydryl group of coenzyme A at a wavelength of 412 nm at 25° C. Assays were initiated by addition of 0.02 ml enzyme extract and were done in triplicate. Specific activity (SA) of malate synthase equals units of enzyme activity per mg of protein. One enzyme unit is defined as the amount of enzyme that produced 1 μmol of product per minute. Total protein concentration was determined by the method used by Lowry, *et al.* (1951).

To detect possible phenolic interference, malate synthase activity was first determined for the individual species. Next, the homogenate from a dark spored species was combined with the homogenate of a light spored species, and the specific activity of malate synthase (the observed SA) was determined for the combined homogenate. If no interfering phenolic compounds were present, the observed SA of malate synthase for the combined homogenate should equal the expected SA, which is the sum of the individual activities of the two species combined divided by two. (The sum is divided by two since mixing

two species dilutes the proteins (enzymes) by half, and assays were still initiated with 0.02 ml of homogenate.) If phenolic interference occurs, the observed specific activity of malate synthase for the combined homogenate should be significantly less than the expected SA.

The homogenates of different light spored species were also combined and assayed. These tests served as controls since the SA of malate synthase of light spored agarics was high and assumed not to be inhibited by phenolic contaminants. The homogenates of different dark spored species were also combined and assayed. These homogenates also served as controls. If the basis of the low SA of malate synthase in the dark spored species (Ruch, *et al.*, 1991; Ruch and Samuel, 1992) is due to phenolic contamination, then the observed SA for the combined dark spored homogenate is expected to be significantly lower than the expected SA due to the abundance of phenolics.

Statistical analyses were done using the Mann-Whitney *U*-test (Zar, 1974) and the chi square test (Sokal and Rohlf, 1981). For the Mann-Whitney *U*-test, the specific activity for all light and dark spored species from all experiments (Tables 2-5) were used. For species used in different assays, such as *Lepiota americana* (Tables 2, 3, 5), each assay was considered to be a separate sample.

RESULTS

The specific activities (SA) of malate synthase for individual and combined homogenates are summarized in Tables 2 through 5. Each table contains the results of all assays run at one time. For species used in assays run at different times, such as *Agaricus campestris* (Tables 2, 4, and 5), the results in each table represent the SA of malate synthase determined for that species during that particular assay; they do not represent an average SA for all assays run at different times in which the species was used. In all cases, the observed SA of malate synthase for combined homogenates (i.e., light plus light spored agarics, dark plus dark spored agarics, and light plus dark spored agarics) were practically identical to the expected SA (Tables 2-5).

Chi square analysis for the observed SA versus expected SA are summarized in Table 6. Chi squares were calculated for the combined results (i.e., light plus light spore agarics in Tables 2 through 5 combined; dark plus dark spored agarics in Tables 3 through 5 combined; and light plus dark spored agarics in Tables 2 through 5 combined). Chi square values were very low ($P > 0.995$), supporting the hypothesis that the observed SAs are equal to (or not significantly different from) the expected SAs.

DISCUSSION

The observed specific activity (SA) of malate synthase was not significantly different from the expected SA of malate synthase in all tests and was supported by a chi square analysis of the observed and expected SA ($P > 0.995$; Table 6). If the homogenization of dark agaric spores had released (or produced) significant amounts of phenolic contaminants, then these homogenates, when

Table 2. Malate synthase specific activity for individual and combined homogenates of agarics.

Species	Malate Synthase SA[b]
Light Spored	
Lepiota americana[a]	8.83
Armillariella mellea	6.69
Pluteus granularis[a]	3.84
Tricholomopsis platyphylla[a]	3.65
Cortinarius sp.[a]	2.75
Dark Spored	
Agaricus campestris	1.79

Homogenates Combined	Malate Synthase SA	
	Observed	Expected[c]
Light Spored x Light Spored		
L. americana x *A. mellea*	7.47	7.76
Dark Spored x Light Spored		
A. campestris x *L. americana*	5.15	5.31
A. campestris x *A. mellea*	4.07	4.24

[a] Species that have not previously been assayed for malate synthase activity.
[b] Specific activity (SA) of malate synthase equals units of enzyme activity per mg of protein. One enzyme unit is defined as the amount of enzyme that produced 1 μmol of product per min.
[c] Expected SA was determined by adding the SA of malate synthase for both species whose homogenates were combined and dividing by two (see Materials and Methods).

mixed with homogenates from light spored species, would have denatured the malate synthase in the mixture. Had this occurred, the observed SA of malate synthase for any of the light spored plus dark spored combined homogenates would have been significantly less than the expected SA (Smith and Berry, 1976). Since the experimental SA's were not reduced, no phenolic contamination occurred. This conclusion is supported by the findings for the two control groups (e.g. light plus light spored agarics and dark plus dark spored agarics). When homogenates of two different light spored species were mixed, the observed SA was not significantly different from the expected SA (Tables 2-5). This result was expected since the SA of malate synthase of light spored agarics was high and assumed not to be inhibited by phenolic contaminants. Likewise, when homogenates of two dark spored species were mixed, the observed SA was not significantly different from the expected SA (Tables 3-5). These findings were unexpected if the basis of the low SA of malate synthase in the dark spored species was due to phenolic contamination. If phenolic contamination was influencing maltate

Table 3. Malate synthase specific activity for individual and combined homogenates of agarics.

Species	Malate Synthase SA[b]	
Light Spored		
Gymnopilus liquiritiae[a]	8.15	
Lactarius psammicola var. *glaba*[a]	6.63	
Lepiota americana[a]	7.91	
Mycena leaiana[a]	4.35	
Dark Spored		
Agrocybe dura[a]	2.75	
Coprinus micaceus	1.41	
Panaeolus foenisecii[a]	2.06	
Homogenates Combined	Malate Synthase SA Observed	Expected[c]
Light Spored x Light Spored		
M. leaiana x *L. americana*	5.90	6.13
L. psammicola x *M. leaiana*	5.37	5.49
Dark Spored x Dark Spored		
C. micaceus x *P. foenisecii*	1.75	1.74
Dark Spored x Light Spored		
C. micaceus x *M. leaiana*	2.72	2.88
C. micaceus x *L. psammicola*	3.93	4.02
P. foenisecii x *L. psammicola*	4.20	4.35
P. foenisecii x *M. leaiana*	3.23	3.21

[a] Species that have not previously been assayed for malate synthase activity.
[b] Specific activity (SA) of malate synthase equals units of enzyme activity per mg of protein. One enzyme unit is defined as the amount of enzyme that produced 1 μmol of product per min.
[c] Expected SA was determined by adding the SA of malate synthase for both species whose homogenates were combined and dividing by two (see Material and Methods).

synthase activity, the observed SA for the combined dark spored homogenate would be predicted to be significantly lower than the expected SA due to the abundance of phenolic contaminants interfering with enzyme activity. Furthermore, Ruch, *et al.* (1991) and Ruch and Samuel (1992) did not observe a similar correlation between light and dark spored species of nongilled mushrooms. The dark spored Aphyllophorales and Gasteromycetes possessed high malate synthase activity, equal to that of the light spored species, suggesting the absence of phenolic contamination.

The absence of phenolic interference implies that the difference in specific activity of malate synthase between dark and light spored agarics is a taxo-

Table 4. Malate synthase specific activity for individual and combined homogenates of agarics.

Species	Malate Synthase SA[b]
Light Spored	
Armillariella tabescens	5.83
Laccaria ochropurpurea	5.10
Lentinellus ursinus[a]	14.79
Leucoagaricus naucinus	5.90
Oudemansiella radicata	2.87
Phyllotopsis nidulans[a]	11.61
Tricholomopsis platyphylla[a]	4.35
Dark Spored	
Agaricus campestris	0.77
Coprinus insignus[a]	1.03
Psathyrella velutina[a]	0.74
Stropharia ambigua[a]	2.09

Homogenates Combined	Malate Synthase SA	
	Observed	Expected[c]
Light Spored x Light Spored		
A. tabescens x *T. platyphylla*	4.89	5.09
P. nidulans x *L. ochropurpurea*	8.13	8.36
Dark Spored x Dark Spored		
P. velutina x *A. campestris*	0.75	0.76
P. velutina x *S. ambigua*	1.36	1.42
Dark Spored x Light Spored		
C. insignus x *L. naucinus*	3.33	3.47
C. insignus x *O. radicata*	1.76	1.95
A. campestris x *A. tabescens*	3.17	3.30
A. campestris x *L. ochropurpurea*	2.98	2.94
S. ambigua x *P. nidulans*	6.71	6.85
P. velutina x *L. ochropurpurea*	2.93	2.92

[a] Species that have not previously been assayed for malate synthase activity.

[b] Specific activity (SA) of malate synthase equals units of enzyme activity per mg of protein. One enzyme unit is defined as the amount of enzyme that produced 1 μmol of product per min.

[c] Expected SA was determined by adding the SA of malate synthase for both species whose homogenates were combined and dividing by two (see Material and Methods).

Table 5. Malate synthase specific activity for individual and combined homogenates of agarics.

Species	Malate Synthase SA[b]	
Light Spored		
Flammulina velutipes[a]	4.24	
Laccaria ochropurpurea[a]	6.21	
Leucoagaricus naucinus	7.92	
Lepiota *americana*[a]	6.72	
Dark Spored		
Coprinus micaceus	2.67	
Agaricus campestris	2.68	
Homogenates Combined	Malate Synthase SA Observed	Expected[c]
Light Spored x Light Spored		
F. velutipes x *L. ochropurpurea*	5.32	5.23
L. naucinus x *L. americana*	7.22	7.32
F. velutipes x *L. naucinus*	5.69	6.08
Dark Spored x Dark Spored		
C. micaceus x *A. campestris*	2.66	2.68
Dark Spored x Light Spored		
C. micaceus x *L. ochropurpurea*	4.31	4.44
C. micaceus x *L. naucinus*	5.03	5.30
C. micaceus x *L. americana*	4.46	4.70
A. campestris x *L. ochropurpurea*	4.48	4.45
A. campestris x *F. velutipes*	3.36	3.46
A. campestris x *L. americana*	4.47	4.70

[a] Species that have not previously been assayed for malate synthase activity.

[b] Specific activity (SA) of malate synthase equals units of enzyme activity per mg of protein. One enzyme unit is defined as the amount of enzyme that produced 1 μmol of product per min.

[c] Expected SA was determined by adding the SA of malate synthase for both species whose homogenates were combined and dividing by two (see Material and Methods).

Table 6. Chi squares for observed specific activity versus expected specific activity of malate synthase.

nomic phenomenon, a fact supported by the good correlation between the findings of this study and the current phylogenetic scheme for the gilled mushrooms. The classical approach to gill mushroom classification, based primarily on morphological, ultrastructural, and biochemical characteristics, separates the light spored agarics into one major lineage and the dark spored agarics into another lineage (Bruns, *et al.*, 1991; Cain, 1972; Moore-Landecker, 1996; Rayner, *et al.*,

Table 6. Chi squares for observed specific activity versus expected specific activity of malate synthase.

	df[a]	Chi Square	*P*
Dark x Dark	3	0.000	> 0.995
Light x Light	7	0.125	> 0.995
Light x Dark	17	0.400	> 0.995

[a] *df* = Degrees of freedom.

1987; Singer, 1986; Tehler, 1988). Recent molecular evidence, using data derived from a comparison of 18S rRNA, 25S rDNA, and mitochondrial rDNA, clearly supports much of the classical view of classification (Bruns, *et al.*, 1991; Bruns, *et al.*, 1993; Johnson, 1997; Moncalvo, *et al.*, 1997; Tehler, 1988; Vilgalys, *et al.*, 1993). In fact Moncalvo, *et al.* (1997), using data derived from a comparison of 25S rDNA, reported that the split of light (or pale) spored agarics into one clade and dark spored agarics into a separate clade had a bootstrap value greater than 70.

A notable exception to the pale spored/dark spored split is *Coprinus comatus* S.F. Gray (Section *Coprinus*, subsection *Coprinus*; Smith, 1986). Although having black spores, *C. comatus*, based on 25S rDNA sequencing information, appears to be more closely related to species of the white spored family Lepiotaceae (Johnson, 1997; Moncalvo, *et al.*, 1997). *Coprinus comatus* was not available for the current study, but it would be interesting to ascertain whether its SA for malate synthase is high like light spored agarics or low like dark spored agarics.

Finally, as noted in previous reports (Ruch, *et al.*, 1991; Ruch and Samuel, 1992), the difference in malate synthase SA between the light and dark spored agarics used in this study was statistically significant as shown by the Mann-Whitney *U*-test ($U = 199.5$; $P < 0.001$; $n_1 = 20$, $n_2 = 10$).

ACKNOWLEDGMENTS

The authors wish to thank the Office of Academic Research and Sponsored Programs, Ball State University, for financial support. We also express our appreciation to Dr. Carolyn Vann for use of equipment in her laboratory.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 201-211

AERIAL CENSUSING OF WHITE-TAILED DEER AND COMPARISON TO SEX-AGE-KILL POPULATION ESTIMATES IN NORTHERN INDIANA

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ABSTRACT: Accurate estimates of population size and composition are important for effective deer management. White-tailed deer (*Odocoileus virginianus*) population estimates were obtained in two northern Indiana Counties, Marshall and Steuben, by winter aerial censusing in 1990 and 1991. To optimize helicopter flight time and decrease the variance among sample areas, stratified random sampling with optimal allocation was used to census 25% of each County. Ground truthing a subsample of woodlots provided a check on the accuracy of the aerial counts. Mean posthunt deer density calculated from aerial counts was 0.09 deer/ha in Steuben County in 1990 and 1991. In 1991, mean deer density in Marshall County was 0.04/ha. Compared to combined ground counts, the aerial counts were 93% accurate. Stratified counts from a helicopter were relatively accurate for northern Indiana when adequate snow cover was present. Population estimates from aerial counts approximated the sex-age-kill model estimates.

KEYWORDS: Aerial censusing, ground truthing, population estimates, sex-age-kill model, white-tailed deer.

INTRODUCTION

Effective management of deer populations requires accurate estimates of population size and composition. Managers want to maintain a healthy deer herd while preventing overpopulation and subsequent habitat damage. Before harvest levels can be decided, the size and composition of the herd and/or any growth trend in the deer population must be determined (Conner, *et al.*, 1986). Small differences in doe harvest rates markedly affect the direction and magnitude of population change in response to hunting (Downing, 1981). Without accurate population estimates, harvest management must proceed conservatively, leaving more individuals than may be required for maintaining a healthy and stable population. At the time of this research, the Indiana Division of Fish and Wildlife was using the sex-age-kill method (Eberhardt, 1960) to estimate deer population size; the accuracy of this method has not been tested within the State.

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Aerial surveying has been used under various conditions to census cervids (Siniff and Skoog, 1964; Bartmann, *et al.,* 1986; Beasom, *et al.*, 1986; Gasaway, *et al.,* 1986; Stoll, *et al.*, 1991). The accuracy of helicopter counts varied tremendously, depending on habitat, terrain, and method. Beasom, *et al.* (1986) reported 33% accuracy in the brushy rangelands of Texas, while accuracy approaching 99% was found for the snow-covered, predominantly agricultural lands in northwestern Ohio (Stoll, *et al.,* 1991). Aerial surveying with proper snow cover (Bartmann, *et al.*, 1986) can provide a reasonably accurate deer count in winter. To determine the accuracy of aerial counts, ground counts should be conducted for comparison (Pollock and Kendall, 1987; deCalesta and Witmer, 1990; Stoll, *et al.,* 1991). Our goal was to obtain independent deer population estimates from both aerial and ground counts and then to compare these counts to Indiana Division of Fish and Wildlife deer density estimates for two northern Indiana Counties made using the sex-age-kill model.

MATERIALS AND METHODS

Marshall and Steuben Counties were selected as the two study areas because they are highly likely to be snow covered in winter; the Counties differed in percent forest cover, degree of woodland fragmentation, and mean deer density. Steuben County is located in the northeastern corner of Indiana, and Marshall County is in north-central Indiana. Both Counties are part of the Northern Lakes Natural Region of Indiana (Homoya, *et al.,* 1984). The dominant trees in this Natural Region are oaks and hickories. Red oak (*Quercus rubra*), white oak (*Q. alba*), black oak (*Q. velutina*), shagbark hickory (*Carya ovata*), and pignut hickory (*C. glabra*) are dominants in the dry and dry-mesic upland forests; beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), black maple (*A. nigrum*), and tulip tree (*Liriodendron tulipifera*) are dominant at mesic sites (Homoya, *et al.,* 1984). Approximately 18% of Steuben County is comprised of forested fragments; many lakes, creeks, and other wetland areas occur as well. Approximately 11% of Marshall County is forested, and the County has a much smaller area covered by lakes, creeks, and wetlands. Woodlots in Steuben County tend to be larger and more contiguous than those in Marshall County. Row crops (corn and soybeans) and hay/pasture fields are the dominant plant communities in both Counties. Mean deer density (estimated by the sex-age-kill model from harvest data) for 1987-1989 was 0.10 deer/ha (0.04/ac) in Steuben County and 0.06 deer/ha (0.02/ac) in Marshall County.

Aerial Surveying. Aerial censusing of diverse habitats can be both bias-prone and expensive. Rental costs during 1990-1991 ranged from $180/hr for the Indiana Division of Fish and Wildlife helicopter to $750/hr for a commercial helicopter and pilot. Total census costs are substantial because they include both the time needed to conduct the surveys and the air time to and from the study areas. Ground counts were conducted to estimate the accuracy of the aerial counts (Stoll, *et al.*,1991). Stratified random quadrat sampling with opti-

mum allocation was used to decrease variance among sample areas and to best utilize flight time (Siniff and Skoog, 1964; Gasaway, *et al.*, 1986).

Twenty-five percent of each County was censused, and both were divided into 2.6-km^2 (1-mi^2) quadrats following the section lines on U.S. Geological Survey 7.5 minute topographic maps. Section lines often run along county roads and are easily seen from the air. We assumed that deer densities were related to the amount of forest cover available and used the percent forest cover to stratify the quadrats. Percent forest cover in each quadrat was determined from the topographic maps and digitized into a mainframe Intergraph to calculate percent forest cover. Current aerial photographs were used to verify and correct U.S. Geological Survey woodlot data. Quadrats were grouped according to percent forest cover into three strata: low (0-9%), medium (10-19%), and high (20-100%). The boundary limits of the strata were set to reflect the range of available percent forest cover present in quadrats (*e.g.*, percent forest cover per quadrat ranged from 0% to 81% and averaged 18% for Steuben and ranged from 0% to 46% and averaged 11% for Marshall County). Optimum allocation was used to determine the number of quadrats selected for censusing among strata (Siniff and Skoog, 1964). Optimum allocation requires more sampling effort in the strata containing more variation (*i.e.*, more sampling was done in the quadrats with more variation in forest cover). Quadrats to be sampled were selected randomly. If a selected quadrat fell within an incorporated town, it was deleted and another quadrat was selected.

A 4-passenger Jet Ranger helicopter flown at an altitude of 60 to 100 m at 35-75 km/hr was used to conduct the surveys. To insure complete quadrat coverage, at least four transects, approximately 406 m apart, were flown. Search time per quadrat varied from 5 to 10 minutes and was concentrated over forested areas. The crew consisted of a pilot, navigator, and two observers. The navigator not only was responsible for directing travel so as to decrease flight time between sampling units but also for assisting in locating groups of deer. Observers, seated on opposite sides of the helicopter, were in continuous audio contact and were responsible for counting the number of deer seen within 203 m (666 ft) of the flight line. The helicopter conducted turns at the end of each transect so that the inside observer could maintain visual contact with any deer seen. To avoid recounting deer, the observers relayed number of deer and their position in the quadrat to each other. The total number of deer in each quadrat was the sum of deer from each observer's independent count corrected for those deer seen by both observers.

Aerial censuses were conducted when snow cover was greater than 8 cm and weather conditions provided clear visibility. The accuracy of the aerial surveys was checked by ground counts. Random selection of woodlots for ground truthing was not practical because of problems in obtaining authorized access. The number of woodlots ground truthed in each County was limited by the number of woodlots that could be censused in one day. Woodlots for ground truthing were not selected based on their habitat characteristics or ease of aerial surveying

but solely on their isolation from adjoining census quadrats, ease of access, and their overall configuration and size. Isolation of woodlots from adjoining census quadrats was important to avoid movement of deer from a ground count woodlot into quadrats that were to be aerially censused later. A ground crew was in position at the woodlot before the helicopter arrived. After the helicopter count, the drivers began their count and the helicopter took up a position that allowed its occupants to watch for deer leaving the area. Drivers, spaced not more than 20 m apart, walked through woodlots while blowing whistles and shouting to encourage the deer to flee. The drivers maintained a straight line by radio communication and counted deer that passed back through the line between them and the person to their right. Counters positioned at the sides and end boundaries of the woodlot counted all deer that fled the area ahead of the progressing drive line. Comparing ground counts with aerial counts provided an estimate of aerial count accuracy and allowed for calculation of a sightability correction factor.

Statistical Methods. Differences in mean deer densities within strata by time of day and among days were analyzed using Duncan's Multiple Range Test ($\alpha = 0.05$; SAS, 1988). Pearson's coefficient of correlation was used to evaluate the relationship between percent forest cover and deer density. Deer population estimates were calculated using the procedures outlined by Cochran (1977) and Davis (1982) and illustrated by Siniff and Skoog (1964) and Gasaway, *et al.* (1986) for stratified random sampling; allocation of sampling was optimized using Neyman allocation (Cochran, 1977).

RESULTS

Aerial Surveying. In Steuben County, 51 quadrats out of a possible 108 in the high stratum, 20 of 130 in the medium, and 10 of 82 in the low were selected for censusing. In Marshall County, 25 of 46 quadrats in the high stratum, 30 of 132 in the medium, and 55 of 263 in the low were selected for censusing.

In 1990, inadequate snow cover in Marshall County prevented aerial censusing. Aerial censusing in Steuben County was conducted on three days in February. Snow depth on the first day was acceptable, but snow-cover conditions deteriorated with each successive day so that by the third day bare ground was visible in the open fields. A total of 77 quadrats (24% of the County) was censused. The number of quadrats censused for the high, medium, and low strata were 48, 19, and 10, respectively. A total of 1,743 deer was observed. The high stratum had a greater mean number of deer per hectare than the other strata; the low stratum had a higher mean than the medium stratum (Table 1). Variance within strata was mixed, but only in the low stratum was the variance significantly different between days; the variance increased with each successive day of censusing as snow cover declined. The mean number of deer seen also decreased with declining snow cover.

In the winter of 1991, weather conditions allowed censusing of 25% of both Steuben and Marshall Counties. Portions of Marshall County were surveyed

Table 1. Summary data from aerial censuses of white-tailed deer in Steuben and Marshall Counties, Indiana, in the winters of 1990 and 1991.

County, Year	Stratum	Number of % Quadrats Censused	Forest Cover / ha		Deer / ha	
			$\bar{X}$	SE	$\bar{X}$	SE
Steuben, 1990	High	48	29.37	0.013	0.0991	0.0192
	Medium	19	15.74	0.006	0.0624	0.0163
	Low	10	7.04	0.004	0.0819	0.0275
Marshall, 1991	High	25	28.52	0.015	0.0907	0.0218
	Medium	30	14.62	0.006	0.0576	0.0095
	Low	55	5.77	0.003	0.0237	0.0048
Steuben, 1991	High	51	30.02	0.013	0.1014	0.0137
	Medium	20	15.58	0.006	0.0948	0.0257
	Low	10	6.74	0.005	0.0375	0.0180

on five days in January. Snow conditions did not allow surveys to be conducted on consecutive days. A total of 1,372 deer was observed in Marshall County. Steuben County was surveyed on 31 January and 1 February by flying the same quadrats as in 1990, and 1,927 deer were observed. For both Counties in 1991, the high strata had the highest mean number of deer, and the medium strata had a higher number than the low strata (Table 1). Further, no significant differences in number of deer was seen between days or between morning and afternoon surveys.

Percent forest cover was significantly correlated ($r = 0.33$, $P < 0.001$) with the number of deer counted in each quadrat. In Steuben County, r was 0.21 in 1990 ($P = 0.034$) and 0.24 in 1991 ($P = 0.016$). In Marshall County in 1991, r was 0.41 ($P < 0.001$).

Ground Counts. Ground counts were conducted in five woodlots in Steuben County on 27 February 1990 and in six woodlots on 31 January 1991 with a ground crew of 14 and 13 people, respectively. Helicopter observers missed 3 of 100 deer in 1990 and 16 of 123 deer in 1991. Ground counts were conducted in four woodlots in Marshall County on 24 January 1991 by a crew of 16 people. The same number of deer was counted by both helicopter and ground surveys. Combining the ground counts for both Counties resulted in an average accuracy of 93% and a correction factor of 1.08 for aerial counts.

Population Estimation. The County population estimate was calculated by multiplying the sum of the weighted, stratified means per quadrat by the total number of quadrats in the County (Burgdorf, 1991). The sightability correction factor was calculated by combining the ground counts from both Counties.

Surveys conducted in Steuben County in 1990 resulted in a population estimate of 6,678 deer, which, when multiplied by the combined sightability cor-

Table 2. Comparison of white-tailed deer population estimates calculated from aerial censuses and by the sex-age-kill method in Indiana in 1990-1991.

County, Year	Preharvest Sex-Age-Kill Population Estimate	Legal Harvest	Adjusted Sex-Age-Kill Population Estimates	Aerial Census Population Estimates (95% C.I.)
Steuben, 1990	9,456	2,818	6,638	7,212 ($5,550 < \bar{x} < 8,924$)
Marshall, 1991	7,114	2,180	4,934	5,005 ($4,136 < \bar{x} < 5,874$)
Steuben, 1991	8,143	2,892	5,251	7,436 ($5,601 < \bar{x} < 9,271$)

rection factor (1.08), yielded a corrected, total estimated posthunt, prior to recruitment, deer population for Steuben County (Table 2) of 7,212 deer or 0.09 deer/ha total area (0.04/ac). The 95 percent confidence interval was 7,212 ± 1,712 deer. In 1991, aerial surveys in Steuben County resulted in a posthunt deer population estimate of 6,886 deer, which yielded in a total corrected population estimate of 7,436 deer or 0.09 deer/ha total area (0.04/ac). The 95 percent confidence interval was 7,436 ± 1,835 deer. Aerial surveying in Marshall County in 1991 resulted in a posthunt deer population estimate of 4,635 deer and a corrected total posthunt population estimate of 5,005 deer or 0.04 deer/ha total area (0.02/ac). The 95 percent confidence interval was 5,005 ± 869 deer.

To compare the posthunt deer density estimates from the aerial surveys with the preharvest sex-age-kill estimates, the number of deer harvested in the County was subtracted from the preharvest sex-age-kill estimate. The deer density estimates calculated for Marshall and Steuben Counties from aerial surveys compare closely with the Indiana Division of Fish and Wildlife's sex-age-kill estimates (Table 2).

DISCUSSION

We decided to census 25% of each county based on the results of other studies. Beasom, *et al.* (1986) investigated the accuracy and precision for different sampling intensities in a mesquite-mixed brush habitat in Texas and found that sampling intensity did not significantly affect the mean percentage of deer observed (similar means were observed at 100% sampling intensity as at 50% and 25%). The resulting variance for deer observed in smaller samples was somewhat larger than for total coverage of the area but not large enough to justify the increased cost of helicopter time (Beasom, *et al.*, 1986). Evans (1975) suggested that a negative bias in the total number of deer seen results when the sampling intensity increases above 25% of the area.

In an effort to decrease variance among sample areas and optimize flight time, we followed the examples of Siniff and Skoog (1964) and Gasaway, *et al.* (1986) for stratified random quadrat sampling with optimal allocation (Neyman allocation). Stratification groups the more homogeneous quadrats together so that within strata variances are relatively small (Siniff and Skoog, 1964; Gasaway, *et al.*,1986). Knowledge of the total possible number of quadrats grouped in each stratum and the variance within each stratum is essential to allocating sampling effort optimally. Frequently, estimates of relative density will approximate measures of relative variance. Because we lacked knowledge of deer density distributions in the Counties, we need a measure of relative habitat suitability as a surrogate measure of relative variance (Gasaway, *et al.*,1986). Although deer in Indiana do not usually exhibit yarding behavior in winter, they do gather in small herds of 20 or so animals and tend to frequent forested areas or other vegetative cover (Mumford and Whitaker, 1982). Optimum allocation, which requires more sampling effort in the strata containing more variation, was used to determine the number of quadrats selected for censusing among strata (Siniff and Skoog, 1964; Cochran, 1977). During the 1990 winter survey in Steuben County, a large number of deer were observed in low stratum quadrats on the third day, presumably because of a lack of snow in the open fields while snow cover still existed in the woodlots. This large number of deer using open fields was contrary to the assumption that deer would seek shelter in the woodlots and affected the mean number of deer calculated for the low stratum in Steuben County in 1990.

In aerial surveys, visibility bias resulting from the failure to observe all animals is considered the primary source of error (Samuel and Pollock, 1981). Many factors, such as weather, terrain, vegetation, and pilot/observer fatigue, can exacerbate the inherent visibility bias of aerial counts (Siniff and Skoog, 1964; Pollock and Kendall, 1987). In the brush-covered rangelands of Texas, Beasom, *et al.* (1986) found that helicopter surveys of white-tailed deer were 26-40% accurate. In large enclosures of snow-covered pinyon (*Pinus edulis*)-juniper (*Juniperus osteosperma*) woodlands in Colorado, between 60% and 68% of the mule deer (*Odocoileus hemionus*) were seen during helicopter counts (Bartmann, *et al.*, 1986). Rice and Harder (1977) conducted a mark-recapture study with helicopter surveys of white-tailed deer and found that accuracy ranged from 51% to 70% of the animals known to be present (127/km^2) in a 122-ha enclosure in northern Ohio with snow present on the various brush, field, and hardwood habitat types. In western Ohio, where forested area ranged between 4% and 14% and deer densities ranged between 2 and 5 deer/km^2, helicopter counts were 99% accurate (Stoll, *et al.*, 1991). The Ohio study is the one most similar to ours in the amount of forest cover, snow cover, terrain (predominantly level) , woodlot size (small), and deer densities (low). As Stoll, *et al.* (1991) stated, this combination of factors is probably what allows aerial censusing to achieve such high accuracy. The accuracy of our aerial survey ranged from 86% to 100%. The accuracy of the aerial counts was affected by the incomplete

coverage and duration of the snow cover, especially during the first winter. A mottled background, caused by the lack of complete snow cover, makes observations more difficult and, therefore, less accurate (Siniff and Skoog, 1964).

The ground counts were a reliable check of the helicopter surveys. Although our woodlots were not chosen randomly, they were chosen without knowledge of habitat structure (*e.g.*, ease of visibility was not a factor in the selection process). Some important rules for the drive-line censusing of deer are: 1) the understory and terrain of the area must allow for optimal viewing between drivers; 2) persons in the drive line must be sufficiently close together; and 3) the line must be kept straight (deCalesta and Witmer, 1990). Recommended between-driver distances are 40 m (Hosely, 1936), 15-30 m (Trippensee, 1948), and 22 m (deCalesta and Witmer, 1990). The personnel manning our drive lines were less than 20 m apart and maintained a straight line through the sparse understory by using radio headsets for communication. Woodlots censused ranged from 9 to 28 ha in size. The small woodlot size, level terrain, sparse understory vegetation, adequate personnel, and snow-covered fields as boundaries allowed for reliable ground counts of the deer. Although the potential existed for missing deer (*e.g.*, those that remained bedded), we believe this bias was small.

The correction factor for forested areas was applied to the total deer estimate calculated from the aerial counts. The correction factor was not applied to the mean number of deer in only the high and medium forest cover quadrats; it was also applied to the low forest cover quadrats. Even though many deer were counted in the open fields of Steuben County in 1990, the sightability correction factor was used. The sightability correction factor was low enough that it did not substantially inflate deer population estimates.

Deer density in each stratum was closer to the anticipated pattern (high stratum > medium stratum > low stratum) in 1991 than in 1990, possibly due to better snow cover and the increased experience of both the observers and pilots. LeResche and Rausch (1974) found that experienced observers saw significantly more deer than did inexperienced observers.

In both Counties, a positive correlation existed between percent forest cover and deer density, although the coefficient of correlation was low. This correlation suggests that forest cover alone does not necessarily reflect deer presence. Individual forest stands vary in quality as deer habitat; other habitats, such as Conservation Reserve Program lands, may also be used by deer in winter. Steuben County has more than 11,000 ha (27,000 ac) registered in the Conservation Reserve Program compared to 1,994 ha (4,927 ac) in Marshall County. According to Indiana Division of Fish and Wildlife biologists, Conservation Reserve Program lands are important habitat for deer in winter. Lack of Conservation Reserve Program lands in Marshall County might be the reason for the lower variances in the numbers of deer per quadrat calculated for all strata in Marshall County in contrast to the higher variances in Steuben County. The presence of Conservation Reserve Program land was not considered during stratification within Counties. Including Conservation Reserve Program lands in the

stratification process could improve mean estimates by decreasing the variance within strata.

Linear fence rows and roads, generally with north-south and east-west orientations, helped distinguish the boundaries of quadrats so that their limits could be ascertained. Care was taken not to count deer outside the quadrat boundary lines, but a source of error was the inability of observers to accurately count large groups of animals. Large herds were circled with the helicopter in a clockwise manner so that the navigator and one observer were able to simultaneously count until their counts agreed or the deer groups dispersed. Other factors also affected deer presence or absence in woodlots—the presence of free-roaming dogs, recent timber harvests, and the juxtaposition of the woodlots to urbanized areas. However, these factors were not sufficiently monitored to allow evaluation of their effects in this study.

The total deer population estimates calculated for Marshall and Steuben Counties by the Indiana Division of Fish and Wildlife's sex-age-kill method are similar to the estimates generated by the aerial surveys. Posthunt estimates from preharvest sex-age-kill estimates are likely overestimates of population size, because the crippling loss and nonharvest mortality numbers are not known and are not subtracted from the preharvest sex-age-kill estimates. Helicopter surveys, on the other hand, tend to underestimate deer populations and thus the need for correction factors. Deer population estimates from the sex-age-kill method for Steuben County (1990) and Marshall County (1991) fall within the 95% confidence intervals of the aerial survey estimates. The estimate obtained from the aerial survey of Steuben County in 1991 is higher and does not contain the sex-age-kill estimates within its 95% confidence interval. Change in the rate of population increase could be influenced by the rate of hunting pressure or the effect of weather conditions on the number of deer harvested in 1990. For example, one of the assumptions of the sex-age-kill method is that the annual buck harvest (hunting pressure) is consistent from year to year. Since all assumptions for the sex-age-kill method cannot be fully met, the method may be subject to error. The ability of the sex-age-kill model to reflect yearly variations in hunting pressure and population fluctuations in Indiana should be investigated further.

CONCLUSIONS

Use of forest cover percentage as a surrogate for deer density in the stratification of the Counties may have resulted in lower variances within strata; however, an improved method of stratification (*i.e.*, including other habitat types such as Conservation Reserve Program land) may be more helpful in future surveys. The relatively high accuracy of our helicopter counts can be attributed to the combination of low deer densities, small woodlot size, hardwood stands with virtually no conifers, sparse understory, and open snow-covered fields surrounding woodlots. The sightability correction factor calculated for Marshall and Steuben Counties would not be a valid correction factor for all Indiana counties, because

the habitat and terrain of southern and northern Indiana differ greatly. Marshall and Steuben Counties are more similar to each other than either is to the more forested and hilly terrain common in southern Indiana.

To improve future aerial surveys, we offer a number of recommendations: 1) surveys should be conducted on consecutive days of complete snow cover to decrease the chances of human activities causing deer herd movements between adjacent, counted versus uncounted, quadrats; 2) the acreage of Conservation Reserve Program lands, classified wildlife areas, and so forth, should be considered in stratification; 3) to improve stratification, a nearest-neighbor rating for each quadrat based on the habitat available in adjoining quadrats should be incorporated; 4) practice counts should be made each year in another area prior to starting the actual survey to allow crew members to gain experience; 5) yearly aerial surveys should be conducted during adequate snow cover to investigate the effect of increased harvest rates on population estimates; and 6) the number of ground truth counts should be increased to better measure accuracy.

Aerial surveys produced reliable estimates of deer density in Marshall and Steuben Counties and corresponded well with the estimates made using the sex-age-kill method. Still, the inputs for the sex-age-kill model need to be refined and verified by additional population studies.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to the Indiana Division of Fish and Wildlife and Purdue University for their funding and support and to the many Purdue University student volunteers who participated in the ground truthing counts. This is journal paper number 14372 from the Purdue Agricultural Experiment Station.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 213-231

THE EFFECTS OF WHITE-TAILED DEER ON PLANT COMMUNITIES WITHIN INDIANA STATE PARKS

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ABSTRACT: The white-tailed deer (*Odocoileus virginianus*) thrives in the habitat mosaic currently found in Indiana and may reach population densities that alter the structure and composition of forest ecosystems in the absence of population control through predation. Our research was initiated to examine the relationship of deer browsing to the condition of the plant communities within Indiana State Parks. During the summer of 1996, thirteen Indiana State Parks and five control areas (where deer hunting was permitted) were sampled to evaluate the impact of deer on these plant communities. During the summer of 1997, one park was added, and fourteen parks were sampled. Significant declines (versus control areas) in the percent cover of herbaceous species and the density of woody stems 50-200 cm in height were observed. For example, both measures were significantly less in three parks in 1997, an additional two parks had significant declines in percent cover of herbaceous species, and one park had a significant decline in the density of woody stems. In general, our research suggests that white-tailed deer populations are impacting Indiana State Parks at varying levels and that these impacts are changing the structure and composition of the plant communities therein.

KEYWORDS: Deer browse, garlic mustard, herbivory, *Odocoileus virginianus*, pawpaw.

INTRODUCTION

White-tailed deer (*Odocoileus virginianus*) are highly adaptable and thrive in the agricultural mosaic created by modern land use. In the absence of population regulation by predation, hunting, or disease, populations can quickly increase to numbers exceeding the carrying capacity of their habitat (Anderson, 1997). White-tailed deer had been extirpated in Indiana by the late 1880s as a result of habitat destruction and unregulated harvest, but they were successfully reintroduced during the 1930's and 1940's. Within 15 years of those reintroductions, deer were beginning to cause localized damage to crops and the native vegetation in areas around their release sites. The first modern deer hunt in Indiana was held in 1951 to remove the annual surplus of deer and lessen crop damage (Mumford and Whitaker, 1982; Brown County State Park Deer Study Committee, 1993).

Excessive populations of white-tailed deer can have a profound effect on plant species composition and community structure in forest systems (Deelen,

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et al., 1996; Redding, 1995; Anderson and Katz, 1993; Strole and Anderson, 1992; Balgooyen and Waller, 1995; Frelich and Lorimer, 1985; Anderson and Loucks, 1979; Telfer, 1972). For the purposes of this study, we defined overabundant populations as populations that have reached a level where they are beginning to negatively impact plant communities. In managed systems, the selective foraging habits of white-tailed deer have posed many concerns over stand regeneration after harvest (Redding, 1995; Trumbull, *et al.*, 1989; Marquis, 1974). Selective foraging can cause a shift in herbaceous species composition toward non-palatable species (Bowersox, *et al.*, 1995; McCormick, *et al.*, 1994).

Dramatic understory alterations resulting from excessive deer browsing are becoming more frequent and may increase the possibility of local extirpation of some more preferred plant species (Strole and Anderson, 1992). In Brown County State Park, Parker and Van Kley (1993) found that the high density of deer in the park had virtually removed the subcanopy and greatly reduced the herbaceous ground cover. Little recovery of the understory plants was evident in the spring of 1994 following the removal of approximately 400 deer during December 1993 (Parker and Brown, 1994). Since that time, deer reductions have been initiated in other Indiana State Parks where the effects of excessive deer populations have been observed on plant communities. Herein, we present the results of a study initiated to monitor the impact of white-tailed deer on the plant communities in Indiana State Parks. Specifically, we assess the relationship between high densities of white-tailed deer and plant communities in Indiana State Parks by examining how herbaceous ground cover, woody browse species abundance, and understory species richness (S), evenness (E), and diversity (Shannon-Weiner, H′) have responded.

MATERIALS AND METHODS

Study Area. During May and June 1996, 13 Indiana State Parks were examined to assess the effect of white-tailed deer on plant community condition (Figure 1). These parks are located throughout the State and were selected to provide a range of size, shape, and landscape context. To control for possible regional variations in species and growing conditions, the parks were clustered by Natural Region as defined by Homoya, *et al.* (1985). A single control area where deer hunting was permitted was sampled within each Natural Region. Data collected by the Indiana Department of Natural Resources on the number of deer killed per unit of hunter effort in state parks and fish and wildlife areas (unpublished data) suggest that hunting reduces the density of white-tailed deer (Figure 2). Areas which have been hunted for many years, such as fish and wildlife areas and Charlestown State Park, have lower numbers of deer harvested per hunter-day than do recently hunted state parks. The number of deer harvested per hunter-day is declining in parks where repeated hunting has occurred, such as Brown County, Potato Creek, and Pokagon State Parks.

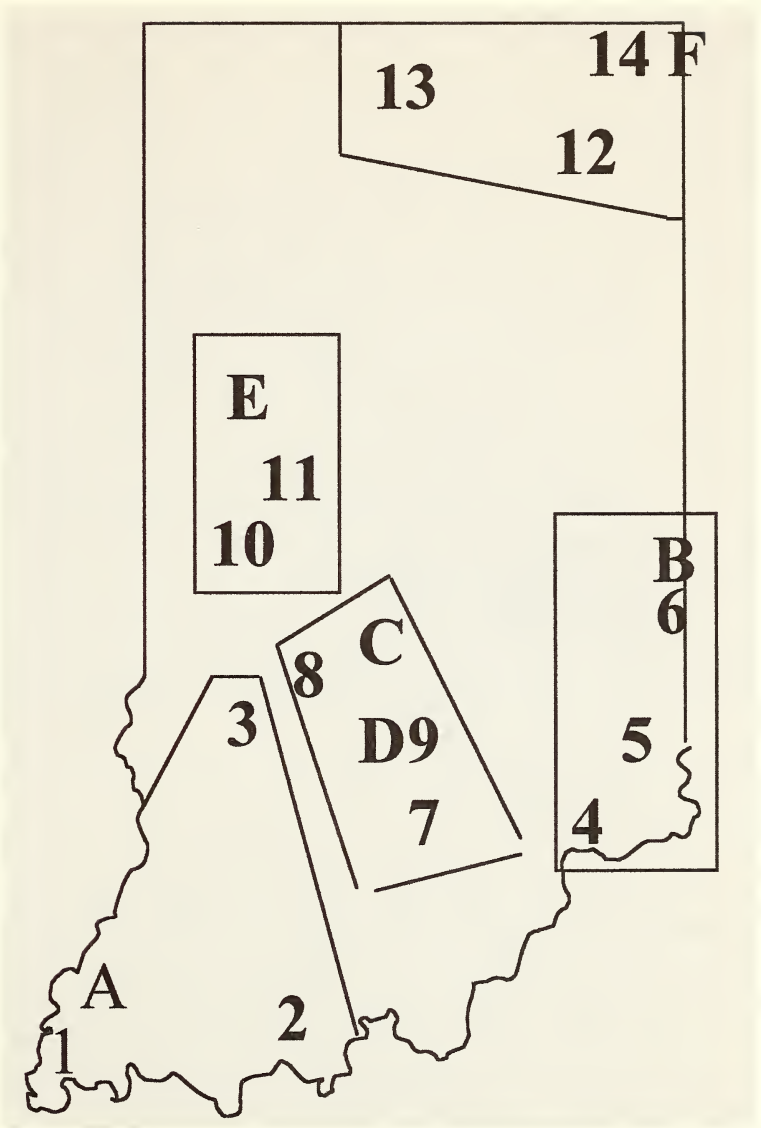

Figure 1. Locations of the Indiana State Parks and control areas studied during 1996 and 1997 by Natural Region: **Southwestern Lowlands Natural Region**, 1 = Harmonie, 2 = Lincoln, 3 = Shakamak State Parks as well as A = Indian Mounds Farm (control); **Bluegrass Natural Region**, 4 = Clifty Falls, 5 = Versailles, and 6 = Whitewater State Parks as well as B = Webster Farm (control); **Highland Rim Natural Region**, 7 = Spring Mill, 8 = McCormick's Creek, and 9 = Brown County State Parks as well as C = Yellowwood State Forest (control) and D = Morgan-Monroe State Forest (control); **Central Till Plain Natural Region**, 10 = Turkey Run and 11 = Shades State Parks as well as E = Martell Experimental Forest (control); **Northern Lakes Natural Region**, 12 = Chain O' Lakes, 13 = Potato Creek, and 14 = Pokagon State Parks as well as F = Oakhill Camp (control). Polygons indicate groupings of parks with a control area based upon a Natural Region.

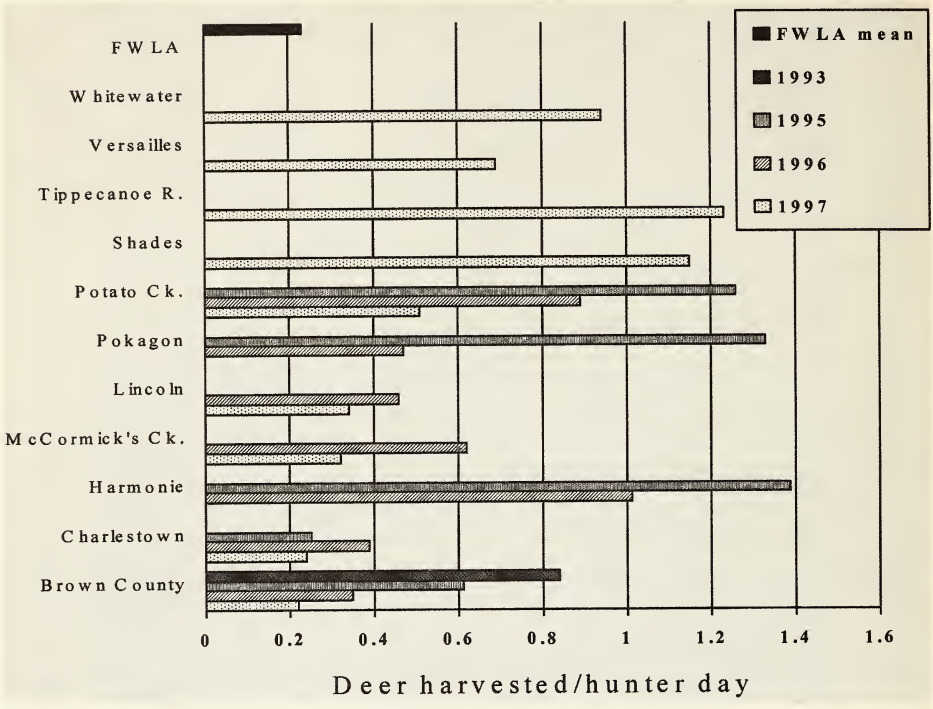

Figure 2. Number of deer harvested per hunter-day for Indiana State Parks that have had deer population reductions and the mean deer harvest per hunter-day on Indiana fish and wildlife areas from 1986 to 1995.

In 1996, six forest stands were randomly selected within the central area of each park using U.S. Geological Survey quad maps and ground reconnaissance. Harmonie, Versailles, Spring Mill, McCormick's Creek, Turkey Run, and Pokagon State Parks all had three existing plots (Brown, 1996), so three additional sites were selected to provide a total of six 300 m^2 circular sample plots per park. All experimental plots were located within mesic communities of closed canopy mature hardwood forest. Aspect, ground flora species, and overstory tree species composition were used to discern mesic sites. Plots were permanently marked with rebar for future monitoring. Similarly, six 300 m^2 circular plots were established in each control area.

During May and June 1997, the above sites were revisited, and one additional park was examined. Five established plots in Brown County State Park (Brown, 1996) were resampled, and one additional plot was established. Three existing plots in Yellowwood State Forest (Brown, 1996) were also sampled.

Field Sampling Design. During May and June 1996 and 1997, we sampled ground flora coverage on line transects running parallel to the contour of the slope on six sample plots per area. On each slope, a base stake was randomly placed at approximately mid-slope to serve as the end of the center 10 m line transect. Two additional 10 m line transects were placed at random distances

(between two and five meters) parallel to the center transect. One of these transects was located upslope from the center transect, and one was located downslope from it. The distance in centimeters of the line transect covered by each herbaceous species was recorded. These distance values were then used to calculate percent cover. The overlap of woody plants less than 50 cm in height was included in this category. The number of woody stems 50 to 200 cm in height was tallied by species on a 50 m^2 circular plot centered at the base stake. The stem tallies provided a density estimate of the number of stems per 50 m^2 for each plot. These tallies were then converted to stems per hectare. The density of woody stems was measured in all the parks sampled in 1996 but was resampled in 1997 only in parks that had deer reductions during the winter of 1996 (Harmonie, Lincoln, McCormick's Creek, Pokagon, and Potato Creek State Parks). Woody stem density was recorded on all the plots in Brown County State Park, which was added in 1997.

Data Analysis. Regression analysis indicated that a significant correlation did not exist between sampling date and mean percent cover ($F_{1,21} = 0.0113$, $P = 0.916$, $R_2 = 0.0005$). Therefore, the means for each park were considered independent. The mean percent cover of the herbaceous species and woody stem densities were then compared for each year between parks and control areas using a one-way ANOVA and Tukey's pairwise multiple comparisons test (Jandel Scientific, 1995). All comparisons were done by Natural Region to control for regional variations in percent cover and woody stem density. The Kolmogorov-Smirnov normality test was used to test the normality for the percent cover and woody stem data (Jandel Scientific, 1995). The data from the majority of parks and controls for all variables were normally distributed (78-100%), so no transformations were used to improve the normality of the data.

Species richness (S), evenness (E), and diversity (Shannon-Weiner, H′) were calculated for each plot using the distance covered by each species in relation to the total transect distance (3000 cm/plot). All herbaceous species and any woody vegetation less than 50 cm in height covering a transect were included in the calculation of the above estimates. The mean values for each park and for its control area were compared using a one-way ANOVA and Tukey's pairwise multiple comparisons test. These comparisons were done by Natural Region to control for regional variation in plant community composition and structure. Sedges (*Carex* spp.) and grasses (Poaceae) were combined into one group for analysis. The Kolmogorov-Smirnov normality test was used to test the assumption of normality. Due to the short duration of this study and the limited sample size, no significant differences were detected between years for any of the factors examined ($p > 0.05$).

The composition of the herbaceous communities was analyzed by computing the percent cover of each individual species encountered in a park during 1997. The species that contributed the most to the total percent cover are reported in the text. These figures illustrate the uneven distribution of plant species within and between sites. The composition of the sapling layer (stems 50-200

cm in height) at each park and control area was analyzed by examining the number of stems of each species per hectare. These data are reported where obvious differences existed. The 1997 data were used because these data were the most recent and were available for all fourteen parks discussed in this paper.

RESULTS

Southwestern Lowlands Natural Region. In 1996, one park (Harmonie State Park) had significantly less herbaceous cover than the control area ($p < 0.05$, Table 1). The differences in other parks were not significant. Woody stem density was not significantly different for any of these parks (Table 2). Mean plot species richness (S), evenness (E), and Shannon-Weiner species diversity (H′) of the parks did not differ significantly from their values in the control area (Tables 3-4).

In 1997, Harmonie State Park was again the only park with significantly less herbaceous cover than the control area ($p < 0.05$, Table 1). Lincoln and Harmonie State Parks did not differ significantly in density of woody stems per hectare from their control area (Table 2). Woody stem density in Shakamak State Park was not recorded during the 1997 field season because no deer reduction was conducted in this park in the winter of 1996. Mean plot species richness, evenness, and diversity of the parks also did not differ significantly from their values in the control area in 1997 (Tables 3-4).

The sapling layer in Harmonie State Park was dominated by papaw (*Asimina triloba*), a species that is not consumed by white-tailed deer. This species may mask differences in woody stem density between the park and its control area. The composition of the sapling layer at Harmonie State Park in comparison to its composition in the control area in 1997 reflected this relationship (Figure 3). The herbaceous layer in the parks sampled was dominated by May apple *(Podophyllum peltatum*), Christmas fern (*Polystichum acrostichoides*), and jack-in-the-pulpit (*Arisaema triphyllum*). May apple accounted for approximately 58% and 38% of the total herbaceous cover in Lincoln and Shakamak State Parks, respectively, but only accounted for 20% of the total herbaceous coverage in the control area. Christmas fern was most common in Shakamak State Park, where it accounted for approximately 13% of the total herbaceous cover. Christmas fern made up approximately 5% of the coverage at the control area. Jack-in-the-pulpit accounted for 17% of the total herbaceous cover in Harmonie State Park and was also quite common in the control area, where it accounted for 18% of the total herbaceous coverage. The extensive coverage of Star-of-Bethlehem (*Ornithogalum umbellatum*) on one plot at Shakamak State Park accounted for approximately 8% of the total percent cover of herbaceous species in the park. This species was not present on sample plots in any of the control areas or any other park.

Bluegrass Natural Region. In 1996, the values for mean percent cover of herbaceous species for Clifty Falls, Whitewater, and Versailles State Parks were not significantly different from their control area (Table 1). However, the den-

Table 1. Comparisons of mean percent cover of herbaceous species between parks and control areas by Natural Region. All tests were done with a one-way ANOVA and Tukey's pairwise multiple comparisons test. An asterisk (*) denotes a significant difference between a park and its control area ($p < 0.05$).

	Mean Percent Cover		Standard Error	
	1996	1997	1996	1997
Southwestern Lowlands Natural Region				
Harmonie State Park	4.7*	6.8*	1.8	2.8
Lincoln State Park	18.6	21.8	3.0	6.3
Shakamak State Park	29.6	35.7	9.8	9.1
Control Area (Indian Mounds Farm)	30.9	31.7	3.2	4.1
Bluegrass Natural Region				
Clifty Falls State Park	24.2	32.4	6.8	9.6
Versailles State Park	32.7	44.6	6.9	7.9
Whitewater State Park	40.2	42.5	6.2	6.7
Control Area (Webster Farm)	31.9	36.4	3.8	4.7
Highland Rim Natural Region				
Spring Mill State Park	38.5	41.5	4.7	4.6
McCormick's Creek State Park	21.3	25.6	7.5	7.4
Brown County State Park		6.3*		1.2
Control Area (Morgan-Monroe State Forest)	45.1	33.6[1]	11.9	4.7
Central Till Plain Natural Region				
Turkey Run State Park	37.7	42.1	3.7	7.0
Shades State Park	39.2	34.6	3.1	1.9
Control Area (Martell Experimental Forest)	47.6	50.5	5.4	5.1
Northern Lakes Natural Region				
Chain O'Lakes State Park	32.7*	28.1*	3.9	4.7
Potato Creek State Park	12.2*	14.8*	2.8	4.1
Pokagon State Park	26.6*	27.7*	1.8	2.8
Control Area (Oakhill Camp)	58.4	48.2	6.4	5.7

[1] In 1997, three plots sampled that year in Yellowwood State Forest were included in the calculation of this mean.

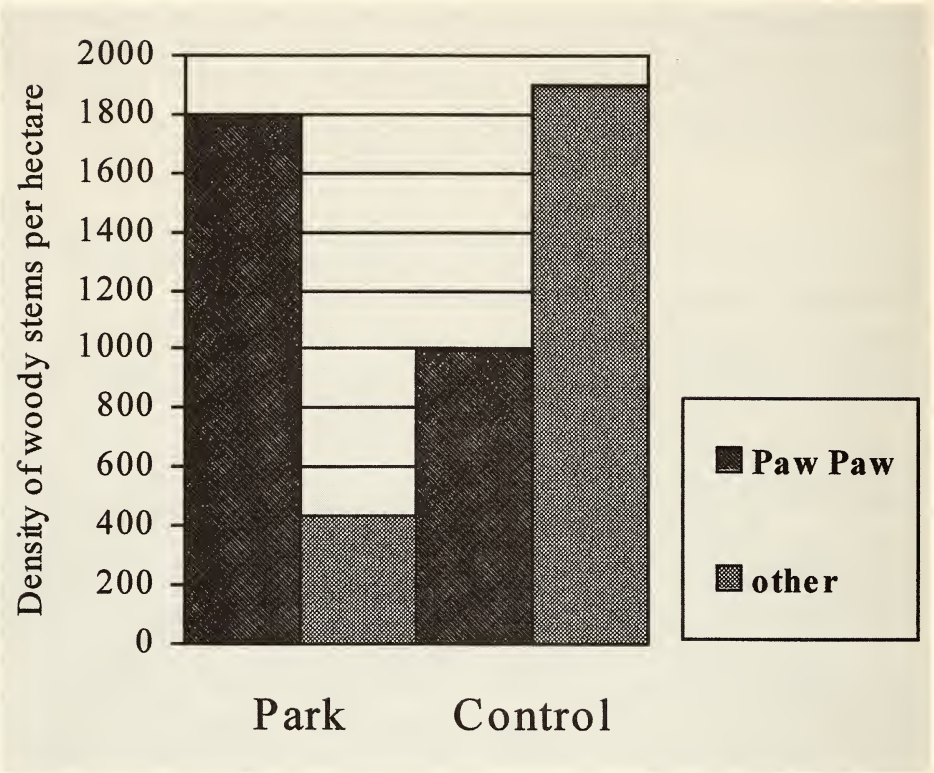

Figure 3. Density of woody stems 50-200 cm in height per hectare of pawpaw versus all other woody species in Harmonie State Park and its control area (Indian Mounds Farm) in May 1997.

sity of woody stems per hectare (50-200 cm in height) within both Whitewater and Versailles State Parks was significantly less than that of the control area ($p < 0.05$, Table 2). Clifty Falls State Park did not display a significant difference (Table 2). The mean plot species richness of Clifty Falls State Park was significantly less than that of the control area ($p < 0.05$, Table 3), but the mean plot species richness of Versailles and Whitewater State Parks did not differ significantly from that of the control area (Table 3). Species evenness and diversity on the sample plots did not differ significantly from the values for the control area for any park in this region (Table 4).

In 1997, no significant differences in percent cover of herbaceous species were found for the parks in the Bluegrass Natural Region (Table 1). Mean plot species richness, evenness, and diversity did not differ significantly from those recorded for the control area (Tables 3-4). Since none of the parks had deer reductions during the winter of 1996, the density of woody stems was not recorded during the 1997 field season.

In Versailles State Park, the herbaceous layer was dominated by narrow-leaved spleenwort (*Athyrium pycnocarpon*), twinleaf (*Jeffersonia diphylla*), gar-

Table 2. Comparisons of mean density of woody stems 50 to 200 cm in height per hectare (MDWS) between parks and control areas by Natural Region. All tests were done using a one-way ANOVA and Tukey's pairwise multiple comparisons test. An asterisk (*) denotes a significant difference between a park and its control area ($p < 0.05$).

	MDWS		Standard Error	
	1996	1997	1996	1997
Southwestern Lowlands Natural Region				
Harmonie State Park	2200	2233	844	967
Lincoln State Park	1267	1000	458	329
Shakamak State Park	1767		418	
Control Area (Indian Mounds Farm)	2667	2900	786	942
Bluegrass Natural Region				
Clifty Falls State Park	5600		1077	
Versailles State Park	1467*		360	
Whitewater State Park	1067*		406	
Control Area (Webster Farm)	8867		2372	
Highland Rim Natural Region				
Spring Mill State Park	3500		1035	
McCormick's Creek State Park	1100*	1200*	184	455
Brown County State Park		667*		146
Control Area (Morgan-Monroe State Forest)	4667	5156[1]	986	1125
Central Till Plain Natural Region				
Turkey Run State Park	2333		1084	
Shades State Park	2200		1141	
Control Area (Martell Experimental Forest)	1200		459	
Northern Lakes Natural Region				
Chain O'Lakes State Park	3066		1455	
Potato Creek State Park	1166*	767*	1047	727
Pokagon State Park	400*	233*	258	158
Control Area (Oakhill Camp)	8366	4767	2705	1204

[1] In 1997, three plots sampled that year in Yellowwood State Forest were included in the calculation of this mean.

lic mustard (*Alliaria officinalis*), and wild ginger (*Asarum canadense*). These species comprised 54% of the total percent cover at the park in comparison to 3% of the total percent cover at the control site in 1997. The mean plot species richness, evenness, and diversity were lower in Versailles State Park than in the control area, but the differences were not significant under the constraints of this study. Garlic mustard accounted for approximately 16% of the total percent cover of herbaceous species in Whitewater State Park but only for 3% of the cover in the control area. Narrow-leaved spleenwort, twinleaf, and wild ginger were not common in the park. At Clifty Falls State Park, wild ginger accounted for approximately 25% of the total percent cover of herbaceous species. This species was not present on transects sampled at the control area but was present on the slopes sampled. May apple accounted for an additional 15% of the total coverage of herbaceous species at Clifty Falls State Park while only accounting for 9% of the total coverage at the control area.

Highland Rim Natural Region. In 1996, Spring Mill and McCormick's Creek State Parks did not display significant differences in mean percent cover of herbaceous species (Table 1). McCormick's Creek State Park had significantly fewer stems per hectare 50-200 cm in height than the control area ($p < 0.05$, Table 2). The density of woody stems per hectare at Spring Mill State Park was not significantly different than that of the control area (Table 2). Mean plot species richness was significantly less in both parks than in the control area ($p < 0.05$, Table 3). The mean plot species evenness and diversity of these parks did not differ significantly from their values in the control area (Table 4).

In 1997, the same situation existed at Spring Mill and McCormick's Creek State Parks as in 1996 (Table 1, 2, 4) with the exception of species richness. The value for richness was slightly higher in the parks in 1997 and no longer significantly different from the richness of the control area ($p > 0.05$, Table 3). Woody stem density was not sampled in Spring Mill State Park during the 1997 field season. Three plots sampled in Yellowwood State Forest in May 1997 were combined with the control plots from Morgan-Monroe State Forest to provide a better representation of the range of variability in this Natural Region. Brown County State Park had significantly less herbaceous cover and fewer woody stems per hectare in 1997 than the control areas ($p < 0.05$, Table 1-2). Mean plot species richness was also significantly different from that of the control areas for this Natural Region ($p < 0.05$, Table 3). Mean plot species diversity and evenness were not significantly different from their values in the control areas (Table 4).

In this Natural Region, wild ginger, May apple, and wood nettle (*Urticastrum divaricatum*) accounted for more of the cover than any other single species in 1997. Wild ginger accounted for 32% of the total herbaceous cover at Spring Mill State Park and for 11% of the total herbaceous cover at McCormick's Creek State Park. This species was not present on any of the transects at Yellowwood State Forest and only accounted for 1% of the herbaceous cover at Morgan-Monroe State Forest. Wood nettle was only recorded at McCormick's Creek State Park, where this species accounted for approximately 20% of the total herba-

Table 3. Comparisons of mean plot understory herbaceous species richness between parks and control areas by Natural Region. All tests were done using a one-way ANOVA and Tukey's pairwise multiple comparisons test. An asterisk (*) denotes a significant difference between a park and its control area ($p < 0.05$).

	Species Richness		Standard Error	
	1996	1997	1996	1997
Southwestern Lowlands Natural Region				
Harmonie State Park	11.0	14.3	3.2	3.6
Lincoln State Park	17.8	18.0	2.8	1.5
Shakamak State Park	16.0	17.3	2.4	1.8
Control Area (Indian Mounds Farm)	17.8	21.8	1.3	1.0
Bluegrass Natural Region				
Clifty Falls State Park	18.7 *	21.8	2.9	2.9
Versailles State Park	23.3	24.5	1.1	1.9
Whitewater State Park	25.5	29.0	1.4	1.1
Control Area (Webster Farm)	29.5	31.5	3.9	3.5
Highland Rim Natural Region				
Spring Mill State Park	20.7 *	21.8	2.1	2.7
McCormick's Creek State Park	19.3 *	21.5	1.6	1.8
Brown County State Park		14.2 *		1.7
Control Area (Morgan-Monroe State Forest)	26.7	26.3 [1]	0.8	1.8
Central Till Plain Natural Region				
Turkey Run State Park	21.2	21.5	3.0	2.4
Shades State Park	26.0	28.3	2.2	3.0
Control Area (Martell Experimental Forest)	22.8	23.0	0.8	0.7
Northern Lakes Natural Region				
Chain O'Lakes State Park	22.0	21.3	1.9	1.7
Potato Creek State Park	12.0 *	15.2	2.0	2.0
Pokagon State Park	20.2	19.8	3.1	3.6
Control Area (Oakhill Camp)	24.8	25.2	2.1	1.5

[1] In 1997, three plots sampled that year in Yellowwood State Forest were included in the calculation of this mean.

ceous cover. May apple was recorded at Spring Mill and McCormick's Creek State Park, where it accounted for approximately 13% and 26% of the total herbaceous cover, respectively. This species was not present on any of the transects in Yellowwood State Forest and only made up 10% of the herbaceous cover at Morgan-Monroe State Forest. The most abundant species by cover in Brown County State Park was jack-in-the-pulpit, which accounted for approximately 14% of the total herbaceous cover. Jack-in-the-pulpit accounted for approximately 5% of the total herbaceous cover in Yellowwood State Forest and 11% of the herbaceous cover in Morgan-Monroe State Forest.

Central Till Plain Natural Region. In 1996, the two parks sampled in this Natural Region (Turkey Run and Shades State Parks) were not significantly different in their percent herbaceous cover from their control area (Table 1). No significant difference was found between these parks and their control area for the density of woody stems (Table 2). The mean plot species richness, evenness, and diversity of the parks in this Natural Region did not differ significantly from their values in their control area (Table 3-4).

In 1997, with the exception of woody stem density (not recorded in 1997 for these parks), the same conditions existed as in 1996. No significant differences in percent cover of herbaceous species were detected (Table 1), and the mean plot species richness, evenness, and diversity did not differ significantly from the values for the control area (Table 3-4).

Christmas fern, May apple, snakeroot spp. (*Sanicula* spp.), and Virginia creeper (*Parthenocissus quinquefolia*) were common in the herbaceous layers of Turkey Run and Shades State Parks. Christmas fern accounted for approximately 8% and 4% of the total herbaceous cover in Turkey Run and Shades State Parks, respectively. This species accounted for < 1% of the herbaceous cover at the control area. May apple was the most abundant species in terms of coverage. This species accounted for approximately 16% and 34% of the total herbaceous cover in these parks while only accounting for 3% of the total herbaceous cover in the control area. Snakeroot spp. were most abundant in Turkey Run State Park, where they comprised 19 % of the total herbaceous cover. This group accounted for 6% of the total herbaceous cover at the control area. Virginia creeper was moderately abundant and made up approximately 4% and 7% of the total herbaceous cover, respectively, in these parks, while accounting for < 1% of the cover in the control area. Wood nettle was common in Shades State Park, where it accounted for 10% of the total herbaceous cover. This species accounted for 2% of the cover in the control area.

Northern Lakes Natural Region. In 1996, all three parks (Chain O' Lakes, Pokagon, and Potato Creek State Parks) in the Northern Lakes Natural Region had significantly lower mean percent covers of herbaceous species than the control area ($p < 0.05$, Table 1). Pokagon and Potato Creek State Parks had significantly fewer woody stems 50-200 cm in height per hectare than the control area ($p < 0.05$, Table 2). In Chain O' Lakes State Park, the mean stem density did not differ significantly from its value in the control area (Table 2). The mean plot

Table 4. Comparisons of mean plot understory species evenness and species diversity (Shannon-Weiner) between parks and control areas by Natural Region. All tests were done using a one-way ANOVA and Tukey's pairwise multiple comparisons test. An asterisk (*) denotes a significant difference between a park and its control area ($p < 0.05$).

	Evenness		Diversity	
	1996	1997	1996	1997
Southwestern Lowlands Natural Region				
Harmonie State Park	0.81	0.78	1.8	1.9
Lincoln State Park	0.60	0.59	1.7	1.7
Shakamak State Park	0.69	0.59	1.8	1.7
Control Area (Indian Mounds Farm)	0.73	0.68	2.1	2.1
Bluegrass Natural Region				
Clifty Falls State Park	0.71	0.71	2.0	2.2
Versailles State Park	0.68	0.68	2.2	2.2
Whitewater State Park	0.70	0.68	2.2	2.3
Control Area (Webster Farm)	0.77	0.80	2.6	2.8
Highland Rim Natural Region				
Spring Mill State Park	0.66	0.66	2.0	2.0*
McCormick's Creek State Park	0.70	0.68	2.1	2.1
Brown County State Park		0.81		2.1
Control Area (Morgan-Monroe State Forest)	0.76	0.80[1]	2.5	2.6[1]
Central Till Plain Natural Region				
Turkey Run State Park	0.68	0.68	2.1	2.1
Shades State Park	0.66	0.65	2.2	2.2
Control Area (Martell Experimental Forest)	0.78	0.75	2.4	2.4
Northern Lakes Natural Region				
Chain O'Lakes State Park	0.77	0.77	2.4	2.3
Potato Creek State Park	0.65	0.68	1.6	1.8
Pokagon State Park	0.56	0.60	1.7	1.8
Control Area (Oakhill Camp)	0.71	0.70	2.3	2.3

[1] In 1997, three plots sampled that year in Yellowwood State Forest were included in the calculation of this mean.

species richness of Potato Creek State Park was significantly less than that of the control area ($p < 0.05$, Table 3). The mean plot species richness of the other parks did not differ significantly from the richness of the control area (Table 3). The mean plot species evenness and diversity of the parks did not differ significantly from the values found at the control area (Table 4). Deer reductions were conducted during the winter of 1996 in Pokagon and Potato Creek State Parks but not in Chain O' Lakes State Park.

In 1997, with the exception of woody stem density in Chain O' Lakes State Park (not recorded in 1997), the same conditions were observed as in 1996 (Tables 1-4). While the herbaceous cover at Pokagon State Park was significantly less than that in the control area, the extent of the reduction in herbaceous cover may have been masked by the extensive coverage of the exotic garlic mustard in this park (Figure 4).

Garlic mustard accounted for approximately 32% of the total herbaceous cover in Pokagon State Park but did not show up on any transects at the control area. May apple was also abundant in these northern parks and accounted for 11%, 29%, and 26% of the total herbaceous cover in Chain O' Lakes, Potato Creek, and Pokagon State Parks, respectively. This species accounted for 9% of the total cover of herbaceous species on the control area.

DISCUSSION

These data suggest that some Indiana State Parks are being negatively impacted by their resident populations of white-tailed deer. Areas where deer hunting historically has been permitted generally have higher mean percent covers of herbaceous species than areas where hunting historically has been prohibited (Figure 5). Hunted areas also tend to have higher densities of woody stems 50-200 cm in height (Figure 5). However, the density of woody stems 50-200 cm in height was quite variable between control areas (Figure 5). This variability may be a result of past disturbance. Therefore, the use of a single control area per Natural Region may not provide a wholly adequate representation of the range of variability present within a given Region, but, in most cases, one control area does provide a reasonable approximation. Taking this limitation into account, several trends were apparent. Deer may not have significantly reduced the diversity or evenness of the herbaceous plant communities in the majority of the parks sampled during this study. However, the deer did significantly reduce the mean percent cover of herbaceous species and the densities of woody stems in several parks. The changes may indicate that either most species are being utilized in proportion to their abundance or that the composition of the herbaceous layer has shifted to less palatable species.

The plant communities are dominated by a few rather common species in each park. These species and species that are known to actually increase as browse intensity increases on the remainder of the plant community (*i.e.*, garlic mustard) may mask the impacts of overbrowsing in its early stages. Garlic mustard is not often consumed by deer and tends to increase in abundance as deer abun-

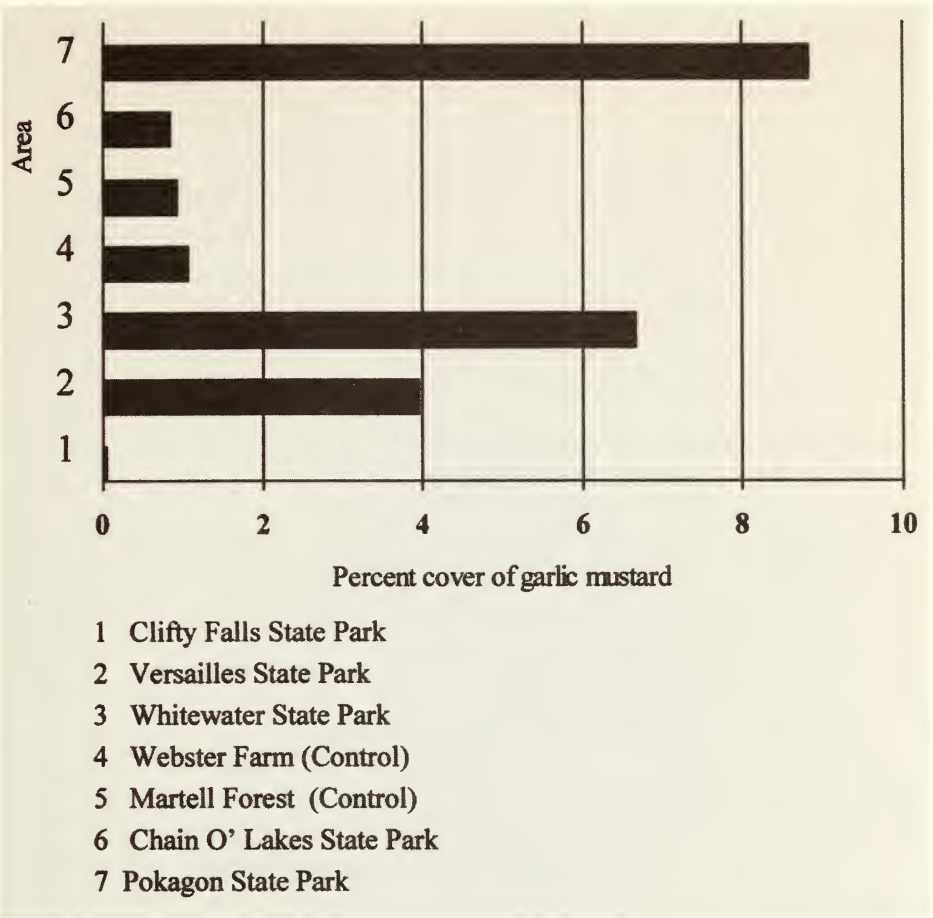

Figure 4. Percent cover of garlic mustard in Indiana State Parks and control areas sampled during May and June 1997.

dance increases (McShea and Rappole, 1997). Several parks across the State have high cover of garlic mustard (Figure 4). However, the differences in cover for garlic mustard are not statistically significant ($p > 0.05$).

Some of the more severely impacted parks, such as Brown County and Harmonie State Parks, appear to attain higher mean plot species evenness and diversity than less impacted parks in their respective Natural Regions. White-tailed deer are selective browsers when resources are abundant, but they switch to a generalist diet when resources become scarce (Nudds, 1980). Therefore, in the most severely impacted parks, deer may have shifted to a generalist foraging strategy and are utilizing the limited plant resources in the park in proportion to each plant's abundance. Further confirmation comes from the patchy distributions of species found in the less severely impacted parks such as Spring Mill, Turkey Run, and Shades State Parks. As deer remove the more preferred species, less preferred species may increase in abundance. The removal of preferred

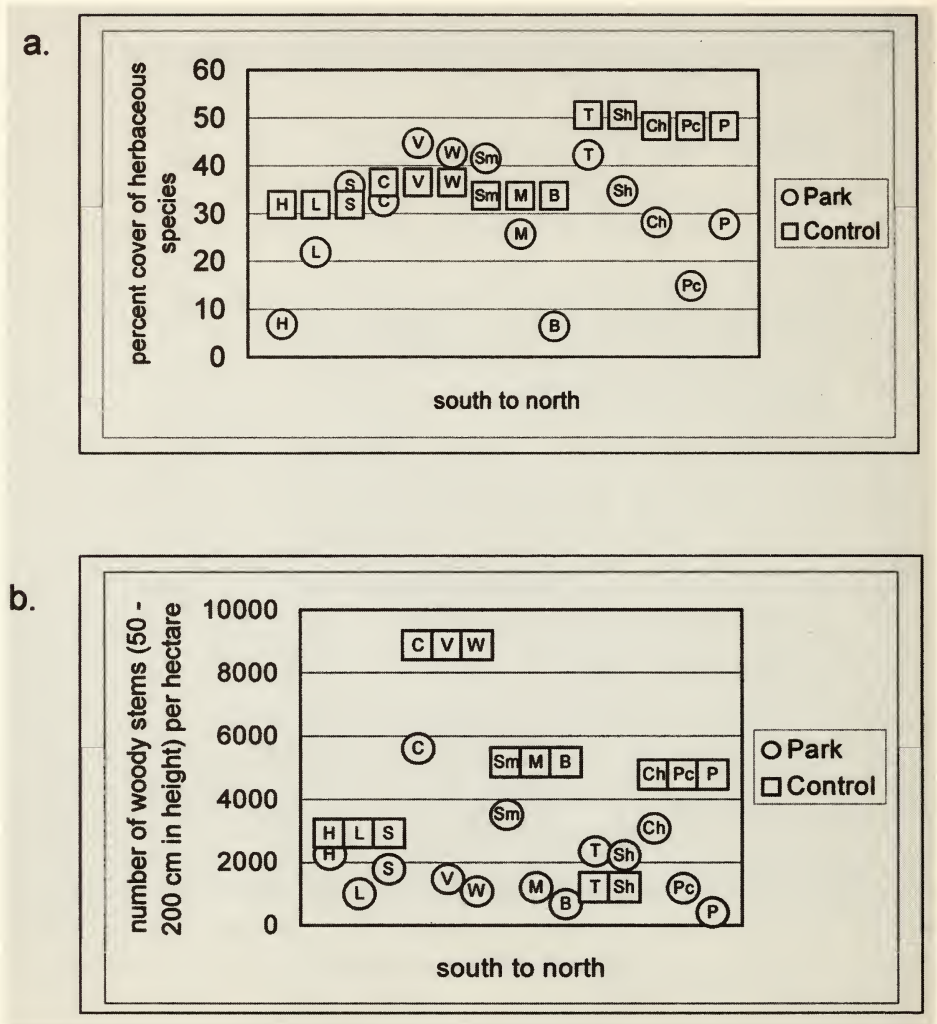

Figure 5. The relationship of the mean percent cover (a) of herbaceous species (1997) and the mean density (b) of woody stems (50-200 cm in height, most recent year of sampling for each area) within the State Parks with those values obtained within corresponding control areas: H = Harmonie, L = Lincoln, S = Shakamak, C = Clifty Falls, V = Versailles, W = Whitewater, Sm = Spring Mill, M = McCormick's Creek, B = Brown County, T = Turkey Run, Sh = Shades, Ch = Chain O' Lakes, Pc = Potato Creek, and P = Pokagon State Parks. The corresponding control areas are indicated by the above code within a square.

species may continue until only less preferred species remain, at which time these species would be eaten in proportion to their respective palatabilities.

The selective use of preferred species by white-tailed deer can lead to the local extirpation of plant species when deer densities are in excess of their carrying capacity (Anderson, 1997). Local extirpation results from disproportion-

ately intense browsing on relatively uncommon species (Strole and Anderson, 1992). Deer have been found to continue consumption of preferred species until those species become very rare before switching to less preferred species (Gillingham and Bunnell, 1989; Westoby, 1974). The significant declines in species richness in five parks were not consistent between years. This variation suggests that species richness may vary on a year-to-year basis. More intense sampling will be needed to determine if species are being lost from the parks due to excessive browsing by deer.

The apparent differences in plant community condition between parks suggests that the parks sampled were being impacted at varying levels. Some parks, such as Spring Mill State Park, are just starting to show evidence of overbrowsing. Other parks, such as Harmonie State Park, showed more evidence. Harmonie had approximately 85% less herbaceous cover than its control area, which is located just outside the Park's boundary. Several variables may lead to the uneven distribution of damage in parks that have not allowed deer hunting for similar lengths of time. Important variables may be the history of deer herd growth in the area, the attitudes of the local people towards poaching, the ease of effective policing of poaching, and the attitudes of the local land owners about legal hunting (Hansen, *et al.*, 1997). Also, the physical attributes of each park—shape, size, and landscape context—may be important. Exclosures have now been established in all the parks sampled and may, over time, improve our understanding of the dynamics of their plant communities.

High densities of white-tailed deer can dramatically alter the species composition of forest communities (Anderson, 1997; Redding, 1995; Strole and Anderson, 1992; Frelich and Lorimer, 1985; Anderson and Loucks, 1979) and negatively impact the populations of other animals, such as song birds (deCalesta, 1994; DeGraaf, *et al.*, 1991). The population levels of deer in Indiana State Parks are influenced by many factors that are beyond the control of resource managers, and the identification of overabundance can be difficult in its early stages. Research is needed to identify the point at which a deer population is beginning to exceed the carrying capacity of its habitat and what predisposes some areas to develop more severe problems with deer overabundance than others.

ACKNOWLEDGMENTS

This research was supported by the Indiana Department of Natural Resources and the Martin Foundation. The authors thank the Divisions of State Parks and of Fish and Wildlife for their assistance in this study. They would also like to thank Jason Meyer and Scott Lawler for their assistance in data collection and entry. Thanks also go to Dr. Robert Swihart for his thoughtful comments on the manuscript during its preparation. Finally, thanks go to Dr. Andrea Easter-Pilcher for her contributions during the initial planning stages of this study. This paper is Purdue Agricultural Station Publication # 15696.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 233-245

SPATIAL DISTRIBUTION OF TRACE METALS IN DELAWARE COUNTY, INDIANA, SURFACE SOILS

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ABSTRACT: Soils in urban areas and in proximity to industrial facilities may become contaminated with trace metals via atmospheric deposition. Soil material was sampled from two depths (0-5 cm and 10-25 cm) within a grid network throughout Center Township, Delaware County, Indiana, and analyzed for concentrations of lead (Pb), cadmium (Cd), zinc (Zn), chromium (Cr), and nickel (Ni). Their concentrations were compared with local and global background levels to determine the extent, if any, of metal contamination from atmospheric deposition. Elevated concentrations of Pb, Cd, and Zn (203.9, 2.5, and 275.2 mg/kg, respectively) were detected, indicating anthropogenic contamination. Lead concentrations were significantly higher ($p < 0.05$) in the surface horizon than in the subsurface. Concentrations of most metals were typically highest near the city center. As a result of sequential chemical extraction of selected soils, the majority of the Pb and Cd was found to occur in residual forms, considered immobile in soil, while Zn, Cr, and Ni occurred in carbonate and organically bound forms, both of which are only slightly bioavailable. These data indicate that the majority of the metals under study pose little, if any, environmental and/or public health hazard from contact with lawns, garden plots, and soil.

KEYWORDS: Atmospheric, cadmium, chromium, deposition, fractionation, lead, nickel, zinc.

INTRODUCTION

Numerous studies (Pichtel, *et al.*, 1997; Maskall, *et al.*, 1996; Kabata-Pendias and Pendias, 1992; Uminska, 1988; Tam, *et al.*, 1985; Czarnowska, *et al.*, 1983; Miller and McFee, 1983) have documented evidence of soil contamination as a result of atmospheric deposition from industrial activity and vehicular emissions. Deposited metals may pose an environmental and/or public health threat, depending on factors such as metal species and the chemical and physical properties of the recipient soils.

Lead battery recycling plants, lead smelters, foundries, and auto-related facilities release lead (Pb), cadmium (Cd), and zinc (Zn) into the biosphere via stack emissions, leaching from waste piles, and dispersion of fugitive emissions (Mattigod and Page, 1983; Förstner, 1995). The use of leaded gasoline was an additional source of atmospheric lead. Although banned in the United States in 1978, leaded gasoline serves as a historical source of soil Pb. Chromium (Cr), nickel (Ni), Cd, and Zn are also released from fossil fuel combustion and from other metallurgical activities, including electroplating.

Dispersion and subsequent accumulation of metals from industry and vehicular emissions can be distributed in various ways. First, a point source of land contamination may occur as a localized elevated concentration surrounded by concentric areas of successively lower concentrations. Second, the distribution can occur as an elliptical area of high concentration along the direction of the prevailing wind (Förstner, 1995; Davies, 1983). Finally, metal concentrations can be distributed vertically (that is, within the soil profile as a function of depth).

In contaminated soils, Pb and Cd compounds are typically concentrated in the upper soil horizons (Hindy, 1991; Buchauer, 1973; Munshower, 1977; Miller, *et al.*, 1983), while Zn, Cr, and Ni compounds tend to be more evenly distributed throughout the profile, indicating a greater mobility in the soil (Little and Martin, 1972). The environmental mobility and bioavailibility of Pb, Cd, Zn, Cr, and Ni in urban soils is dependent upon the physicochemical forms in which the metal is associated in the soil profile (Harrison, *et al.*, 1981). Sequential extraction procedures provide qualitative evidence regarding the forms of the associations of these metals and, thus, of their mobility and bioavailibility (Sposito, *et al.*, 1982; LeClaire, *et al.*, 1984).

The city of Muncie, Indiana, located in Center Township, Delaware County, has been heavily industrialized, housing metal-related facilities for over a century. A systematic soil sampling design was established to determine the average concentrations of Pb, Cd, Zn, Cr, and Ni within the Township. From these data, contour plots were prepared to illustrate the spatial distribution of metal concentrations. These plots were then compared to historic city and road plans and to the prevailing wind direction in order to correlate concentrations with possible sources. The metals were also evaluated for mobility; that is, whether they are retained mostly on the soil surface, or whether they migrate to levels deeper in the soil profile, and the extent to which these metals migrate from a source by way of atmospheric transport.

EXPERIMENTAL METHODS

Center Township (T20N, R10E), Delaware County, Indiana, was divided into 25 equal-sized quadrants (1.2 miles x 1.2 miles). Three replicate soil samples were collected near the center of each quadrant using a 2.5 cm (1 in) diameter stainless steel soil probe. Samples were collected at two depths, 0-5 cm and 10-25 cm, to examine the extent of metal contamination in the shallow soil profile. Sampling was conducted as far as practical from roadways to avoid localized contamination by Pb from the past use of leaded gasoline. Sampling locations included parks, gardens, vacant lots, schoolyards, and churches. In order to assess the presumed background levels of each metal, triplicate samples were taken at both depths approximately three miles north, south, east, and west of Center Township.

Soil samples were prepared for total metal analysis by air drying for 1 week at ambient temperature. The dried samples were crushed and passed through a 2-mm mesh (#10) sieve. Soils were digested for total metals content using a

hot HNO_3 method (Miller, *et al.*, 1983; Harrison, *et al.*, 1981; Ure, 1991; Thompson, 1983). A 0.5 g sample was digested in 10 ml of hot (100° C) 16M HNO_3 for 16 hours in a Hach Digestahl reactor. After overnight digestion, five to eight 1 ml aliquots of 30% H_2O_2 were added to oxidize resistant organic matter. The solutions were filtered into 50 ml volumetric flasks using Whatman #1 filter paper, each extract was brought to volume (50 ml), the extracts were acidified with HNO_3, and they were stored at 4° C until analysis. Concentrations of soluble Pb, Cd, Zn, Cr, and Ni were measured via atomic absorption spectrophotometry (AAS; Perkin-Elmer model 2240, Norwalk, Connecticut; Perkin-Elmer, 1982).

Samples collected in quadrant 18 (SE 1/4, NE 1/4, Sec. 21) had elevated concentrations of all five metals at both depths. Chemical fractionation of soil Pb, Cd, Zn, Cr, and Ni followed the sequential extraction procedure outlined by Sposito, *et al.* (1982). Sposito's method involves sequential extraction of an air-dried soil sample with 0.5M KNO_3 (16h), deionized H_2O (2h; extracted three times and combined), 0.5M NaOH (16h), 0.05M Na_2EDTA (6h), and 4M HNO_3 (16h at 80° C). Metals extracted by KNO_3 and H_2O are assumed to be exchangeable and soluble, respectively. These metals represent the most labile and bioavailable fraction in the soil (LeClaire, *et al.*, 1984). The NaOH is assumed to remove organically bound metals, and EDTA should extract metals from inorganic precipitates. The NaOH and EDTA fractions represent potential reserves of bioavailable metals. Metals removed by HNO_3 are an estimate of residual, nonlabile metals or sulfides (Sposito, *et al.*, 1982). All soil extracts were centrifuged for 15 min. at 3000x g; the supernatant was decanted and analyzed for metal concentrations using AAS.

Soil pH was measured using a pH meter with a glass electrode paired with a calomel electrode. Soil was mixed with deionized H_2O at a 2:1 (v/w) liquid:solid ratio, and the method of McLean (1982) was used.

Total organic carbon (TOC) content was measured on the 0-5 cm samples collected from each quadrant using a loss on ignition method (Nelson and Sommers, 1982). For this method, from 3 g to 5 g of soil (weighed out to 0.0001 g) was placed in a porcelain crucible and dried at 105° C for 1 hour. The sample was then placed in a muffle furnace at 360° C for 2 hours to fully oxidize the organic portion of each sample. Total organic carbon was expressed as the percent of weight lost by ignition in the muffle furnace.

Summary statistics for the metal concentrations in each quadrant were calculated using DATADESK (Data Description, 1997) on a Macintosh Performa 636 personal computer with a Windows operating system. Analyses of variance and *t*-tests were also conducted ($p < 0.05$) using DATADESK.

Contour plots of the mean metal distribution for the 0-5 cm depth were prepared using SURFER (Scientific Software Group, 1994). A grid representing the township was split into 5 rows and 5 columns representing the 25 sampling quadrants. The mean concentration for each metal was entered into the center nodes of each quadrant. Contour plots were developed by SURFER using

a Krieging statistical method. The plots were then exported as .dxf (digital exchange format) and imported into ARCINFO (Environmental Systems Research Institute, Inc., 1994a) to be georeferenced to the actual dimensions of Center Township on a digitized map. This procedure allows for the contour plots to be overlaid onto U.S. Geological Survey topographic maps of the Muncie area using ARCVIEW software (Environmental Systems Research Institute, Inc., 1994b).

RESULTS AND DISCUSSION

The soil pH of the Township samples ranged from 5.9 to 8.0 (Table 1). The average pH was 7.4, which is comparable to pH 6.8 observed for local background samples. The pH for Indiana soils averages 5.5 - 6.0 (U.S. Department of Agriculture, 1972). In east-central Indiana, the predominance of calcitic and dolomitic bedrock results in high carbonate soil types. Czarnowska, *et al.* (1983) noted that soil contamination results in an alkaline soil pH. Soil reaction (*i.e.*, pH) has an effect on the degree of metal solubility and displacement in the soil profile (Czarnowska, *et al.*, 1983). At the pH range observed in Indiana, most metals will tend to precipitate (Kabata-Pendias and Pendias, 1992; Lindsay, 1979). The organic matter content ranged from 2.1% to 10.3% (Table 1) with an average value of 6.1%. The 10.3% organic matter value occurred in quadrant 18 and may be due to the presence of carbonaceous wastes (*i.e.*, slags and/or dense non-aqueous phase liquids).

SPATIAL DISTRIBUTIONS OF SOIL METALS

Lead. The highest surface soil Pb concentrations occur in or near the city center (Figure 1), and in isolated locations, concentrations exceed 460 mg/kg. Soil Pb decreased to 81 mg/kg outside the township limits, which approximates mean local background levels (114.2 mg/kg). Soil Pb concentrations differ significantly ($p < 0.05$) within the surface horizon. These data concur with the results of other studies (Pichtel, *et al.*,1997; Czarnowska, *et al.*, 1992; 1983) that urban Pb concentrations tend to be highest in the city center and/or industrial zones.

The average Pb levels both in the township (Table 1) and local background samples were higher than the global baseline level of 20 mg/kg Pb in surface soils (Kabata-Pendias and Pendias, 1992). Miller and McFee (1983) found that soils in industrialized northwestern Indiana contained 500 mg/kg Pb, several orders of magnitude greater than global baseline levels. Long distance transport from metallurgical industries can significantly increase elemental concentrations in the soil surface (Steinnes, 1984). Certain relatively volatile metals, including Pb, are preferentially concentrated in the smallest particle size fractions occurring in airborne particulate matter (Steinnes, 1983). Additionally, high soil Pb may result from heavy vehicular traffic using leaded gasoline over many decades. High Pb levels were noted along three major roads into Muncie (Figure 1). These roads, running northwest, east, and southwest of downtown, were the original

Table 1. Soil pH, total organic carbon, and total metal concentrations throughout Center Township, Delaware County, Indiana.

Parameter	Depth (cm)	Mean	*p* Value	Max	Min
pH	0-5	7.3 ± 0.6	n/a[1]	8.0	5.9
TOC[2] (%)	0-5	6.1 ± 2.0	n/a	10.3	2.1
Pb (mg/kg)	0-5	203.8 ± 35.9	*[3]	466.3	81.1
	10-25	172.2 ± 28.9		344.8	53.9
Cd (mg/kg)	0-5	2.48 ± 0.2	ns[4]	4.0	1.8
	10-25	2.21 ± 0.2		3.1	1.5
Zn (mg/kg)	0-5	275.2 ± 85.7	ns	2750.0	126.1
	10-25	210.1 ± 37.9		509.6	108.2
Cr (mg/kg)	0-5	9.7 ± 1.4	ns	17.0	4.0
	10-25	8.6 ± 1.4		20.9	3.0
Ni (mg/kg)	0-5	25.0 ± 2.5	ns	49.0	16.7
	10-25	26.8 ± 2.2		42.7	16.7

[1] n/a = Not applicable.
[2] TOC = Total organic carbon.
[3] * = Significant at $p < 0.05$.
[4] ns = Not significant.

thoroughfares into the city. Lead concentrations in roadway soils and rights-of-ways were in the range of 140-540 mg/kg (Ward, *et al.*, 1977; Tam, *et al.*, 1987).

The distribution of Pb in the lower soil horizon (10-25 cm; data not shown) differs significantly ($p < 0.05$) from its distribution in the upper horizon. This difference is presumably the result of the low solubility and thus low mobility of Pb at neutral-high pH (Maskall, *et al.*, 1996; Davies, 1983). The low mobility and high surface retention of Pb in the environment has been documented by a number of researchers (Maskall, *et al.*, 1996; Kabata-Pendias and Pendias, 1992; Harrison, *et al.*, 1981; Davies, 1983). Therefore, Pb will not easily leach into the subsurface nor is it expected to occur in the same pattern as it does on the surface. A variety of factors will influence subsurface Pb concentrations, including soil pH and organic matter content, which varied from 5.9 to 8.0 and 2.1% to 10.3%, respectively (Table 1). Furthermore, soil oxidation-reduction potential and the presence of complexing ions will influence mobility or retention (Kabata-Pendias and Pendias, 1992).

Lead speciation in the surface soil of quadrant 18 was: carbonate > residual >> organically bound > soluble > exchangeable (Figure 2). For the 10-25 cm

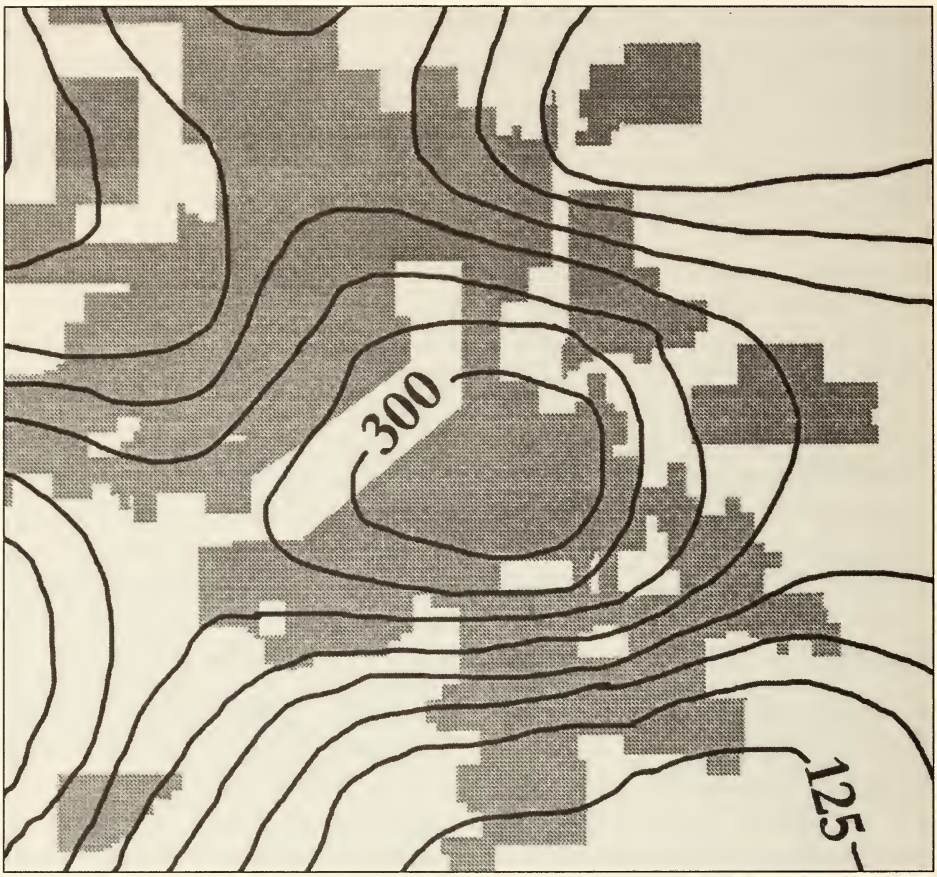

Figure 1. Contour plot of surface soil Pb concentrations (mg/kg; contour interval = 25 mg/kg).

samples, the order was: carbonate >> residual > soluble > organically bound > exchangeable. This order agrees with the results of Pichtel, *et al.* (1997) that carbonate and residual/sulfide are the dominant Pb species in anthropogenically affected soils. High soil pH tends to precipitate Pb as insoluble carbonates, oxides, and hydroxides. Kabata-Pendias and Pendias (1992) also reported the occurrence of Pb carbonates as the dominant Pb species in roadside soils, indicating that anthropogenic Pb should not be very mobile or bioavailable (Maskall, *et al.*, 1996).

Cadmium. Soil Cd levels reached a maximum of 3.2 mg/kg near the city center and decreased to 2.0 mg/kg at the edge of Center Township (Figure 3). The highest Cd readings (4.0 mg/kg) occurred at isolated points in the vicinity of well-established industries. The reported levels are much higher than the local background concentration (0.14 mg/kg). Miller and McFee (1983) found that soils in industrialized northwestern Indiana contained 10 mg/kg Cd, many orders of magnitude greater than background levels. According to Uminska (1993),

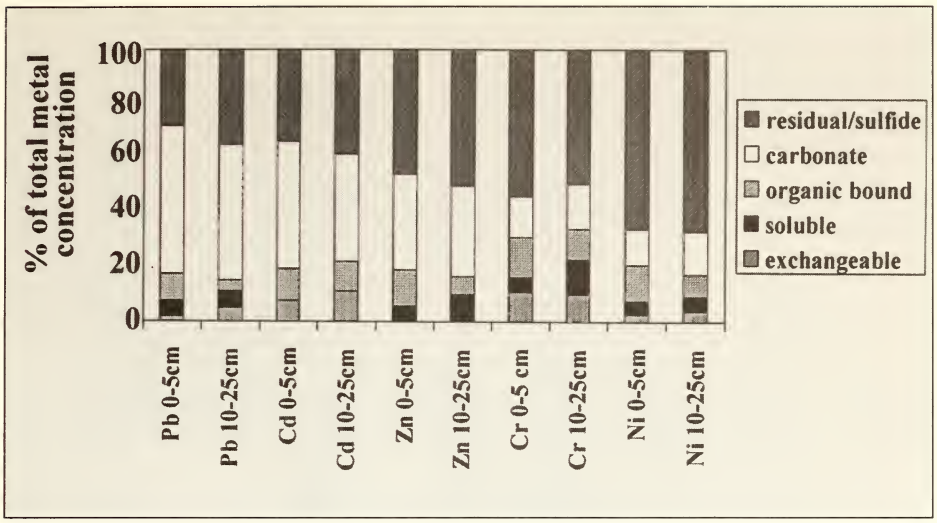

Figure 2. Distribution of soil metal species from a sequential extraction of quadrant 18.

soils with Cd concentrations greater than 1 mg/kg were probably anthropogenically contaminated.

Cadmium in the surface soil occurs primarily as carbonates and follows this order: carbonate > residual/sulfide > organically bound > exchangeable >> soluble (Figure 2). This same order was observed at the lower depth with the exception that carbonate = residual/sulfide. Pichtel, *et al.* (1997) concluded that in areas heavily contaminated with Cd, the greatest proportion of soil Cd occurred as carbonates, organically bound, and exchangeable. Organically-bound Cd is an indication of anthropogenic contamination (Asami, *et al.*, 1995).

The majority of soil Cd is expected to be immobile in the township soils. Mobility of the potentially labile carbonate species is dependent on pH. At pH values above 7.5, any Cd sorbed to soil material is highly immobile (Buchauer, 1973; Hindy, 1991; Kabata-Pendias and Pendias, 1992). Furthermore, sulfide/residual Cd species are immobile in soil.

Zinc. Soil Zn concentrations in the surface horizon ranged from 126 to 2750 mg/kg (Table 1). Local background levels average 126.1 (0-5 cm depth) and 108.2 (10-25 cm), and worldwide concentrations range between 15 and 125 mg/kg (Kabata-Pendias and Pendias, 1992). The highest Zn contours (Figure 4) are located in the area of established industries. Miller and McFee (1983) found that soils in industrialized northwestern Indiana contained 2000 mg/kg Zn, a value1-2 orders of magnitude greater than the background levels. Robberecht, *et al.* (1983) found 2160 mg/kg Zn in soil surrounding a non-ferrous metal industry.

The two Zn distributions (0-5 cm and 10-25 cm) are not significantly different ($p < 0.05$). Zinc is reported to be mobile and equally distributed throughout the soil profile (Kabata-Pendias and Pendias, 1992; Davies, 1983).

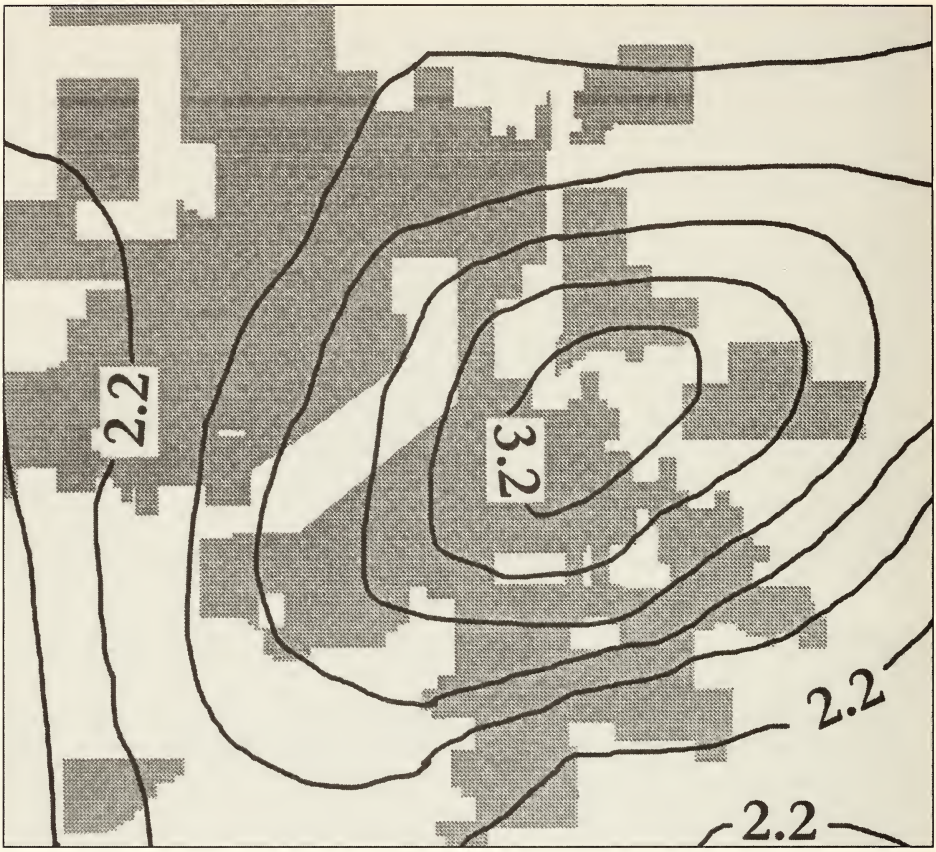

Figure 3. Contour plot of surface soil Cd concentrations (mg/kg; contour interval = 0.2 mg/kg).

Speciation of Zn in the surface soil occurred as: sulfide/residual > carbonate > organically bound > soluble > exchangeable (Figure 2). At the 10-25 cm depth, the order of Zn speciation was: sulfide/residual > carbonate > soluble > organically bound > exchangeable. These results are similar to findings which demonstrated that Zn occurs primarily in the residual/sulfide fraction of contaminated urban soils (Pichtel, *et al.*, 1997). Based on the predominance of the residual fraction, soil Zn is not expected to leach significantly as sulfide/residual species are non-labile. Zinc occurs naturally as ZnS; it also occurs as carbonate precipitates as a result of neutral to high soil pH.

Chromium. Chromium concentrations in Center Township surface soils ranged from 4 to 17 mg/kg (Figure 5, Table 1). Local background levels averaged 9 mg/kg for the surface horizon and 6 mg/kg for the subsurface. The global mean for soil Cr is 54 mg/kg (Kabata-Pendias and Pendias, 1992). Pichtel, *et al.* (1997) measured a maximum of 69 mg/kg in soils from Warsaw, Poland, and Czarnowska, *et al.* (1983) found numerous surface soils in excess of 40 mg/kg.

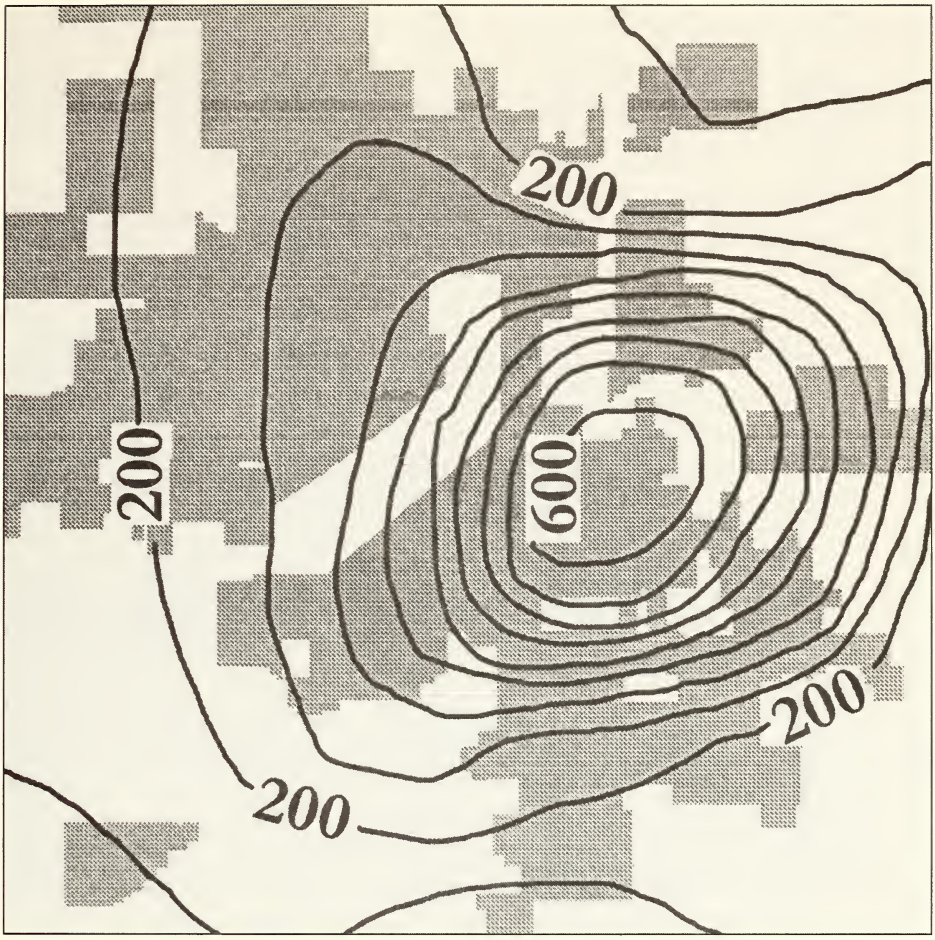

Figure 4. Contour plot of surface soil Zn concentrations (mg/kg; contour interval = 50 mg/kg).

No significant variation occurred among Cr means for either the surface or subsurface, and no significant difference in Cr concentrations was found between the two sampling depths. The average Cr concentration decreased from 9.7 mg/kg to 8.6 mg/kg as the depth increased from 0-5 cm to 10-25 cm.

Surface soil Cr was distributed as: sulfide/residual >> organically bound > exchangeable > carbonate > soluble. The 10-15 cm Cr speciation was: sulfide/residual >> soluble > carbonate > organically bound > exchangeable. The predominance of sulfide/residual Cr in urban soils was also reported by Pichtel, *et al.* (1997). Anthropogenic Cr additions from the atmosphere to soil most commonly result in two Cr species, hydrous oxide bound and organically bound (Kabata-Pendias and Pendias, 1992). Therefore, atmospheric deposition of Cr in Center Township plays a minor role when compared to the presence of residual, non-labile Cr, presumably arising from soil parent material.

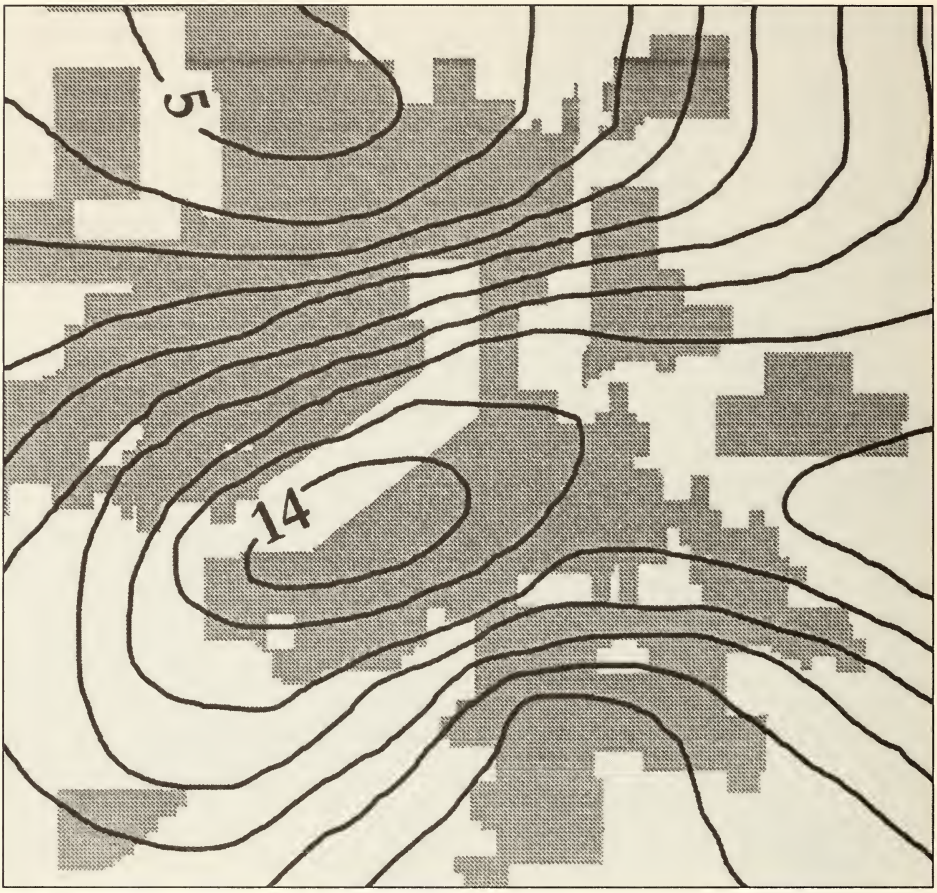

Figure 5. Contour plot of surface soil Cr concentrations (mg/kg; contour interval = 1 mg/kg).

Nickel. Soil Ni concentrations ranged from 32 mg/kg near the downtown to 18 mg/kg south of Muncie (Figure 6). An average Ni concentration of 25.0 mg/kg was observed for the 0-5 cm samples. However, no significant within-group variation occurred. Soil Ni values were lower than local background levels (39.8 mg/kg). Surface soils in the U.S. range in Ni content from < 5 to 150 mg/kg (Kabata-Pendias and Pendias, 1992).

The Ni distribution at the two soil depths was the inverse of all other metals tested in that the greater average Ni concentration (26.8 mg/kg) occurred in the soil collected from the 10-25 cm level; however, the difference between distributions was not significantly different. Nickel concentration often increases with increasing depth in the soil profile (Davies, 1983). As a result of weathering, Ni is easily mobilized. The Ni cation, Ni^{2+}, is stable in an aqueous environment, allowing for migration over long distances in the soil (Kabata-Pendias and Pendias, 1992). The Ni cation may eventually be coprecipitated as Fe and Mn oxides (Sparks, 1995).

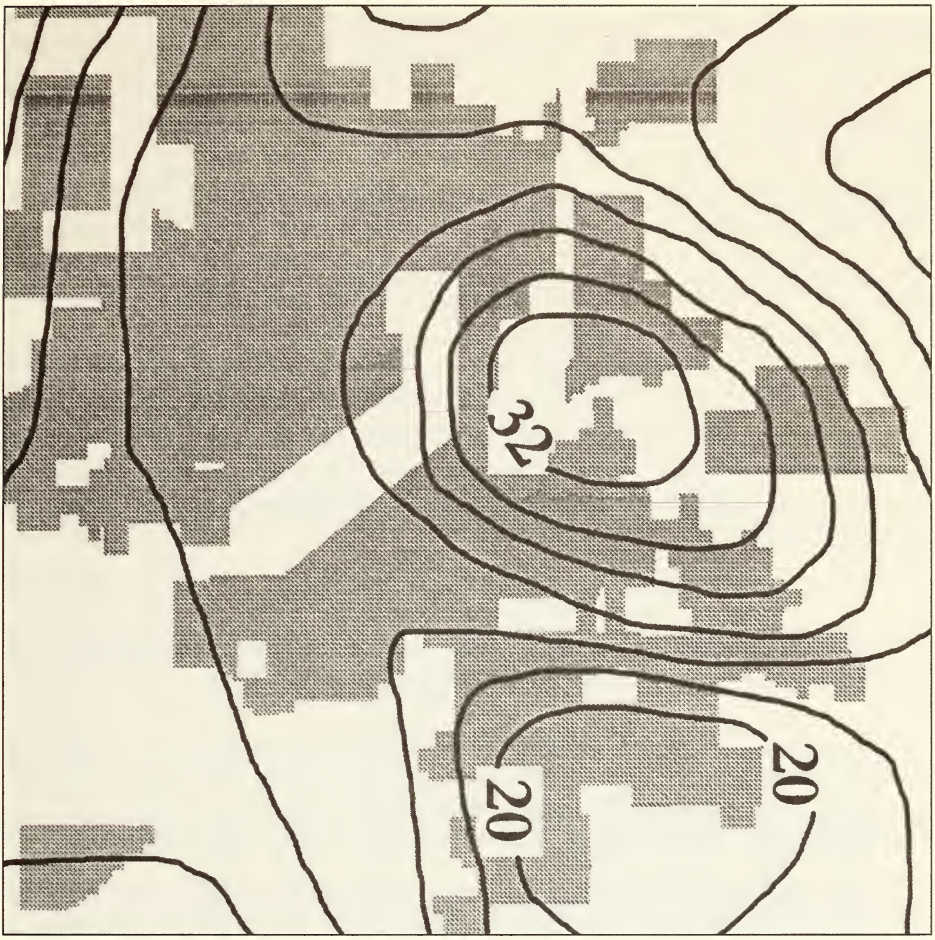

Figure 6. Contour plot of surface soil Ni concentrations (mg/kg; contour interval = 2 mg/kg).

Sequential extraction analysis at the 0-5 cm level resulted in a distribution of: sulfide/residual >> carbonate > organically bound > soluble > exchangeable (Figure 2). This order also held for the 10-25 cm level. Nickel has a strong affinity for sulfur, and in terrestrial rocks, Ni occurs mainly as sulfides, arsenides, and ferromagensium complexes (Kabata-Pendias and Pendias, 1992). Nickel is also associated with carbonates, phosphates, and silicates.

SUMMARY AND CONCLUSIONS

Center Township was assessed for the degree of metal contamination, if any, that resulted from long-term atmospheric deposition. Elevated Pb concentrations were detected as compared to background (*i.e.,* outside township) levels. Levels of soil Cd and Zn were also higher than background values, which indicates anthropogenic contamination. Most of the surface and subsurface contamina-

tion occurs in the city center and in areas of well-established industry. Aside from Pb, metal concentrations did not significantly differ between the two depths.

The minimal leachability of metals in circumneutral pH soils and the tendency of metals to accumulate in the organic material of surface soils is important in terms of potential environmental hazard. Only in highly contaminated soils or in acid, permeable soils are anthropogenic metals found deeper than 5-8 cm below the surface. Once soils become contaminated with metals, they are likely to remain until removed by soil erosion rather than leaching (Davies, 1983). This feature of contaminated soil may have adverse health implications due to human exposure to surface dusts.

As a result of sequential extraction of soils from selected sites, much of the Pb and Cd was found to occur as residual, which is considered immobile in soils, and Zn, Cr, and Ni occurred as carbonate and organically bound forms, both of which are only slightly bioavailable. These data indicate that the majority of the metals measured pose little environmental and/or public health hazard from contact with lawns, garden plots, and soil.

ACKNOWLEDGMENTS

Financial support from the Ball State University Internal Grant Program and the Indiana Academy of Science is gratefully acknowledged.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 247-251

SOME HIGHLIGHTS OF THE CARBONDALE GROUP (PENNSYLVANIAN) IN THE SUBSURFACE IN INDIANA

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ABSTRACT: The Pennsylvanian System in Indiana is composed in ascending order of the Raccoon Creek, Carbondale, and McLeansboro Groups. The Carbondale contains the rocks between the base of the Colchester Coal and the top of the Danville Coal Members and is composed, in ascending order, of the Linton, Petersburg, and Dugger Formations. These formations contain laterally persistent limestone and coal Members that can be traced in the subsurface throughout much of the Illinois Basin. Flooding surfaces are recognized above the Colchester, Houchin Creek, Springfield, and Herrin Coals. The Carbondale ranges in thickness from 270 to 430 feet.

KEYWORDS: Carbondale Group, Pennsylvanian, subsurface Indiana.

INTRODUCTION

The Pennsylvanian System in Indiana is composed of three groups. In ascending order, they are the Raccoon Creek, Carbondale, and McLeansboro Groups (Figure 1). The purpose of this short report is to note the evidence for the rapid flooding of several of the Carbondale coal swamps by basin-wide marine waters and to present a map showing the thickness of Carbondale rocks in the subsurface of Indiana.

GENERAL STRATIGRAPHY

Shaw and Savage (1912) first used the name Carbondale Formation for rocks exposed near Carbondale in Monroe County, Illinois. Kosanke, *et al.* (1960) described the formation in Illinois as the rocks between the base of the Colchester (No. 2) Coal and the top of the Danville (No. 7) Coal. Wier and Gray (1961) defined the Carbondale Group in Indiana as the rocks between the top of the Seelyville Coal Member of the Staunton Formation and the top of the Danville Coal Member of the Dugger Formation. In their report on the Raccoon Creek Group of the Illinois Basin, Droste and Horowitz (in press) proposed that the base of the Colchester Coal instead of the top of the Seelyville Coal be used basin wide to mark the top of the Raccoon Creek Group. In this note, we follow that usage (Figure 1). The Carbondale Group contains, in ascending order, the Linton, Petersburg, and Dugger Formations (Figure 1).

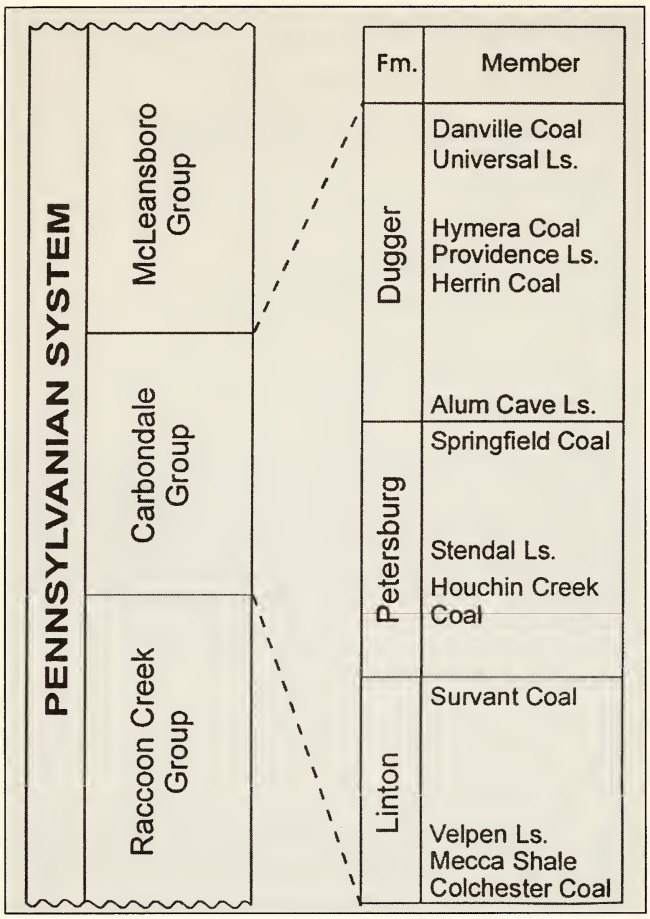

Figure 1. Chart showing the stratigraphic nomenclature used in this report.

Unlike the formations of the Raccoon Creek and McLeansboro Groups, the three Formations of the Carbondale contain several coal and limestone Members that have extensive lateral continuity. Although each of these Members cannot be identified on every log from every well, we are able to identify these selected key members (Figure 1) and their stratigraphic equivalents in the subsurface in Indiana, Illinois, and Kentucky. From one location to another, all of the key coals are replaced by sandstones and all of the key limestones are replaced in one area or another by a fossiliferous shale facies. However, because we can identify the stratigraphic position of these key markers on most logs, we can correlate these selected units in the subsurface throughout most of the Illinois Basin.

COAL AND LIMESTONE COMBINATIONS

A noteworthy feature of Carbondale rocks is that many of the coal members that can be recognized basin wide are overlain by limestone members that also can be recognized basin wide. Consider the combination of the Springfield Coal and Alum Cave Limestone. The Springfield Coal is probably the most often basin-wide identified key marker in the Pennsylvanian System. The Springfield is thick enough in numerous areas throughout the basin to be a very important present and future commercial coal.

An interesting question presents itself. How big was the swamp in which the Springfield peat accumulated? The question has no precise answer because the

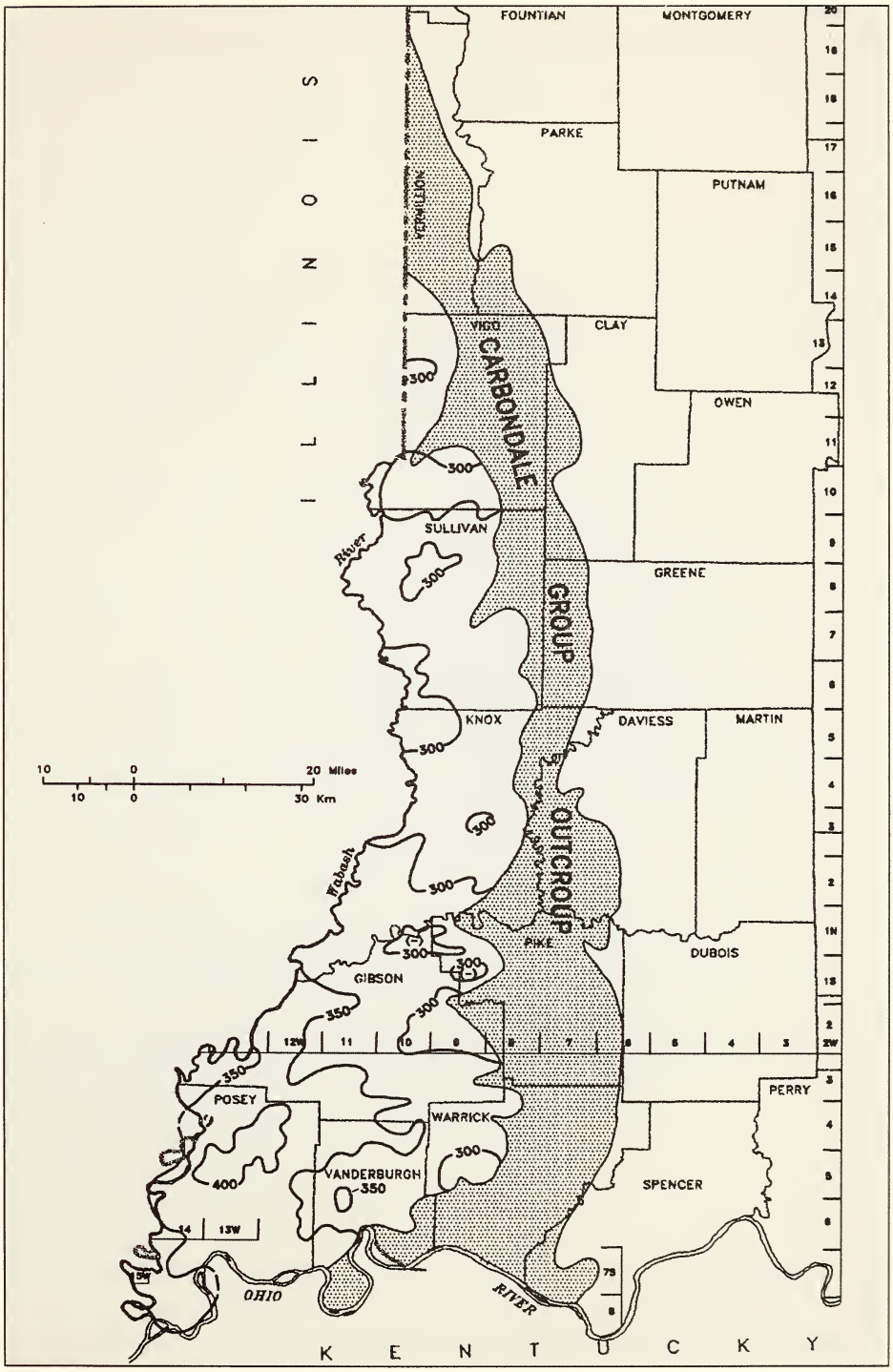

Figure 2. Map showing the thickness of the Carbondale Group in southwestern Indiana. The contour interval is 50 feet.

Springfield occurs at the bedrock surface around the Illinois Basin. Nevertheless, using the eroded limits of the Springfield, we can determine that the Springfield swamp was at least tens of thousands of square miles in area. The entire swamp area was very near sea level, the water table was very high, and the area had very little relief. The interpretive setting was a coastal swamp bounded transitionally on one side by the sea and on the other side by a somewhat higher coastal plain. A few major rivers flowed through the swamp, and these streams transported terrigenous sand and mud from distant inland sources through the swamp to the sea. The location of the rivers can be mapped (Eggert, 1982; Eggert and Adams, 1985). At the stratigraphic position of the Springfield, the coal is replaced by contemporaneous fluvial sediments, channel sandstone, and overbank deposits. This facies relationship explains the earlier statement that every basin-wide coal member is replaced in one area or another by sandstone.

The top of the Springfield Coal is slightly younger eastward. The regional transgressive event (see below) moved across the basin from the south and west to the north and east so that the youngest Springfield Coal is found along the eastward margin of its eroded limit. Although the Springfield Coal does not have the time precision that an ash bed would have, we believe that the top of the Springfield Coal is, within currently available resolution, an approximate time plain in the Illinois Basin.

In many places, several feet of black and very dark gray marine shale separates the Springfield Coal below from the Alum Cave Limestone above. The Alum Cave is a medium- to dark-colored argillaceous limestone containing abundant echinodermal debris and common brachiopod and molluscan fragments. In some places, fusulinids, ostracods, and bryozoans are present. At numerous locations, dark gray fossiliferous shale containing lenses and nodules of limestone occupy the Alum Cave interval. The limestone and associated marine shale of the Alum Cave interval document a fully marine transgression. This basin-wide event records a rapid flooding of the Springfield coal swamp. In the terminology of sequence stratigraphy, a flooding surface overlies the Springfield Coal.

Are there similar events at other stratigraphic positions in the rocks of the Carbondale Group? The interval containing the Mecca (marine) Shale and Velpen Limestone above the Colchester Coal (Figure 1) documents a transgression with a flooding surface above the Colchester. The Stendal Limestone and its Illinois equivalent, the Hanover Limestone, have been correlated with limestones in Oklahoma, Missouri, and Kansas (Wanless, 1957). Clearly, a flooding surface occurs above the Houchin Creek Coal. The Providence Limestone above the Herrin Coal is recognized basinwide and records a flooding surface above the Herrin. Although no basin-wide coal is found below the Universal Limestone (Figure 1), we sugggest that the Universal lies above at least a partial flooding surface.

THICKNESS

Our control is based on well records in the files of the Indiana Geological Survey. For the subsurface study of the Carbondale Group in Indiana, we correlated more than 10,000 well logs. For the thickness map (Figure 2), we used no more than one log per section (*i.e.,* one well per square mile). The Carbondale ranges in thickness from 270 feet to 430 feet. Carbondale rocks in Indiana thicken southward and westward. Northward from central Knox and Daviess Counties (Figure 2), the thickness variation of the Carbondale is small and is within a few 10's of feet of 300. The group exceeds 400 feet in a small depocenter in central and northern Posey County (Figure 2). The southwestward thickening may be a consequence of regional subsidence imposed on variable local rates of subsidence within the basin.

SUMMARY

The Carbondale is much thinner (400 feet) than the Raccoon Creek (1200 feet) or McLeansboro (700 feet) Groups. However, in the rocks of the Carbondale, we see recorded the well-developed basinwide coal and limestone combinations that document several cycles of rapid marine transgression with flooding surfaces above the Colchester, Houchin Creek, Springfield, and Herrin Coals and below the Universal Limestone.

ACKNOWLEDGMENTS

The authors thank J.R. Dodd, H.H. Gray, and S.J. Keller for reviewing an earlier version of this manuscript and very much appreciate the comments of the anonymous reviewers.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 253-255

DAN WIERSMA, PIONEER AGRONOMIST

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Dan Wiersma was born on 4 November 1916 on a farm near Volga, South Dakota. He attended the local rural schools and graduated from Volga High School in 1934. He received his B.S. degree in 1942 from South Dakota State University. Dan was an outstanding athlete both in high school and college. Following graduation, he entered the Army with an ROTC commission and served as a Captain in the European Theater during World War II. Dan was awarded a Purple Heart and Silver Star. After the war, he served as an extension agent in western South Dakota for six years. He then returned to school and earned his M.S. degree from the University of Wyoming in 1952 and his Ph.D. from the University of California in 1956. In graduate school, the future Dr. Wiersma studied plant-soil-water relationships.

Dr. Wiersma (Figure 1) joined the Agronomy Department at Purdue University in 1955 and had research, extension, teaching, and administrative responsibilities. His primary research interests were in irrigation and plant-soil-water relationships. At Purdue, Dan became an effective and highly respected researcher, teacher, and extension specialist. Dan directed research on droughty soils in Indiana and carried out extensive laboratory studies on the water-holding characteristics of over 200 soil types in Indiana. He was the principal authority on irrigation at Purdue. His research supplied the basic information needed by other researchers, crop production specialists, industry agronomists, and State and Federal agencies. In addition, he served on the North Central Regional Committees on irrigation, drainage, soil moisture storage, and the use of water by agronomic crops. Dan was also a member of the Plant-Soil-Water Committee of the National Science Foundation. During parts of 1962-1963, he served with the Rockefeller Foundation's Colombian Program, where he directed irrigation and evapotranspiration research. He was much sought after as a speaker in crop production extension schools, and he also effectively taught a graduate course on plant-soil-water relations.

Dan joined the Indiana Academy of Science in 1960. He was Chair of the Soil Science Section at the Fall Meeting in 1962. Dr. Wiersma published two papers in the *Proceedings*:

Figure 1. Daniel Wiersma in a photograph taken in late 1981 or early 1982 on the occasion of his retirement.

Wiersma, D. 1960. Moisture characteristics of some representative Indiana soil types. *Proc. Indiana Acad. Sci.* 69: 300-304.

_____. 1962. Increasing crop potentials through water availability. *Proc. Indiana Acad. Sci.* 71: 347-352.

In addition, he was co-author of five more papers:

Johannsen, C.J., M.F. Baumgardner, and D. Wiersma. 1971a. Leaf moisture variation within corn leaf sections. *Proc. Indiana Acad. Sci.* 80: 453-460.

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Mannering, J.V. and D. Wiersma. 1970. The effect of rainfall energy on water infiltration into soils. *Proc. Indiana Acad. Sci.* 79: 407-412.

Stivers, R.K., D. Wiersma, and J.G. Flores. 1961. Evaluation of three sources of nitrogen for corn. *Proc. Indiana Acad. Sci.* 70: 217-226.

Turner, R. and D. Wiersma. 1964. Determining evapotranspiration rates and soil moisture levels with climatological data. *Proc. Indiana Acad. Sci.* 72: 244-248.

He was named a Fellow of the Academy in 1977.

Dan became the Director of the Purdue Water Resources Research Council in 1966 and held that office until his retirement 17 years later. Dan was low-key, but he had the ability to meld people of diverse backgrounds in the humanities, science, and engineering into cooperative, enthusiastic, investigative groups. Dan could always recognize fluff and pretense while maintaining a sense of calm and a level of civility and courtesy. He never patronized nor attempted to pre-empt knowledge from an other person's specific discipline. Dan always was alert to opportunity, occasionally locating sources of funding in areas other than his own. He also assisted other faculty members in writing grant proposals and obtaining research funds. Dan's dedication to the water resources program was typified by the modest administrative setting he physically maintained as Director. Rather than having plush quarters and high administrative costs, Dan desired to make as much money as possible available for water resources research. Dan Wiersma had scores friends who appreciate the help he gave them in furthering their careers.

Dr. Wiersma was (Vice-) Chairman of the Governor's Water Resource Study Commission (1980-1982) and, upon completion of the Commission's work, was recognized as a "Sagamore of the Wabash" on 17 June 1983. He was the true author of the Commission's well-recognized book (see Literature Cited), *The Indiana Water Resource: Availability, Uses, and Needs.* As Vice-Chairman, Dan gained the respect and admiration of all the faculty involved in the project due to the exceptionally efficient manner in which he conducted the Commission's business.

Dan was also a devoted family man. He married Esther Sydow of Volga, South Dakota, in 1943. She taught mathematics at the Klondike School in Tippecanoe County, Indiana for several years. Upon retirement, Dan and Esther returned to their birthplace, Volga, where they became heavily involved in community affairs. Dan died in Brookings, South Dakota, on 8 February 1993. Esther survives with two sons, Daniel John of Chanhassen, Minnesota, and Bruce John of Aiken, South Carolina.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 257-266

NEW HARMONY, INDIANA, A CENTURY AND A HALF OF SCIENCE AND ENGINEERING

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ABSTRACT: Events concerning local resource development in the New Harmony area (Posey County, Indiana) are recounted beginning with the communal settlements of the early 1800's and ending with the oil boom prior to World War II. The natural resources and the people who described and used them are highlighted.

KEYWORDS: New Harmony, history, resource development.

INTRODUCTION

Posey County lies in southwestern Indiana and constitutes that corner of the State. The Wabash River forms the County's western boundary with Illinois, and the Ohio River forms its border with Kentucky (Figure 1).

Topographically and geologically, Posey County shows evidence of a turbulent past. The bedrock, either exposed or near the surface, is part of the Pennsylvanian System. Layers of shale, limestone, and sandstone are intermixed with thin coal beds. The major mineable coal seams are deeply buried and are mined today using modern underground methods. The same coal seams lie almost at the surface in Warrick County, where they are mined using surface mining methods. The coal beds dip gently westward below Vanderburgh and Posey Counties toward Illinois.

The bedrock was subjected to faulting following deposition. The Wabash Valley Fault System transects the area from the southwest to the northeast, creating a horst and graben bedrock topography. The age of faulting is post-Pennsylvanian, perhaps Permian, in age (Nelson and Lumm, 1987). The bedrock surface is buried by unconsolidated material ranging in composition from Illinoisan till and Wisconsinan slack-water deposits to recent floodplain deposits and wind-blown soils near the major waterways.

The second to last or Illinoisan glaciation reached Posey County, and the glacier's southern boundary followed the present-day Big Creek drainage in Posey County. Big Creek may have been an old ice-marginal drainage way. During the Wisconsinan or last glaciation, the ice did not extend this far south, but Posey County was significantly impacted by the melting glaciers to the north. The Wabash River served as the major drainage for the region, and high water, either from ice dams or simply excessive runoff from the retreating ice, affected the area. Flooding along the Wabash River caused intermittent backwater

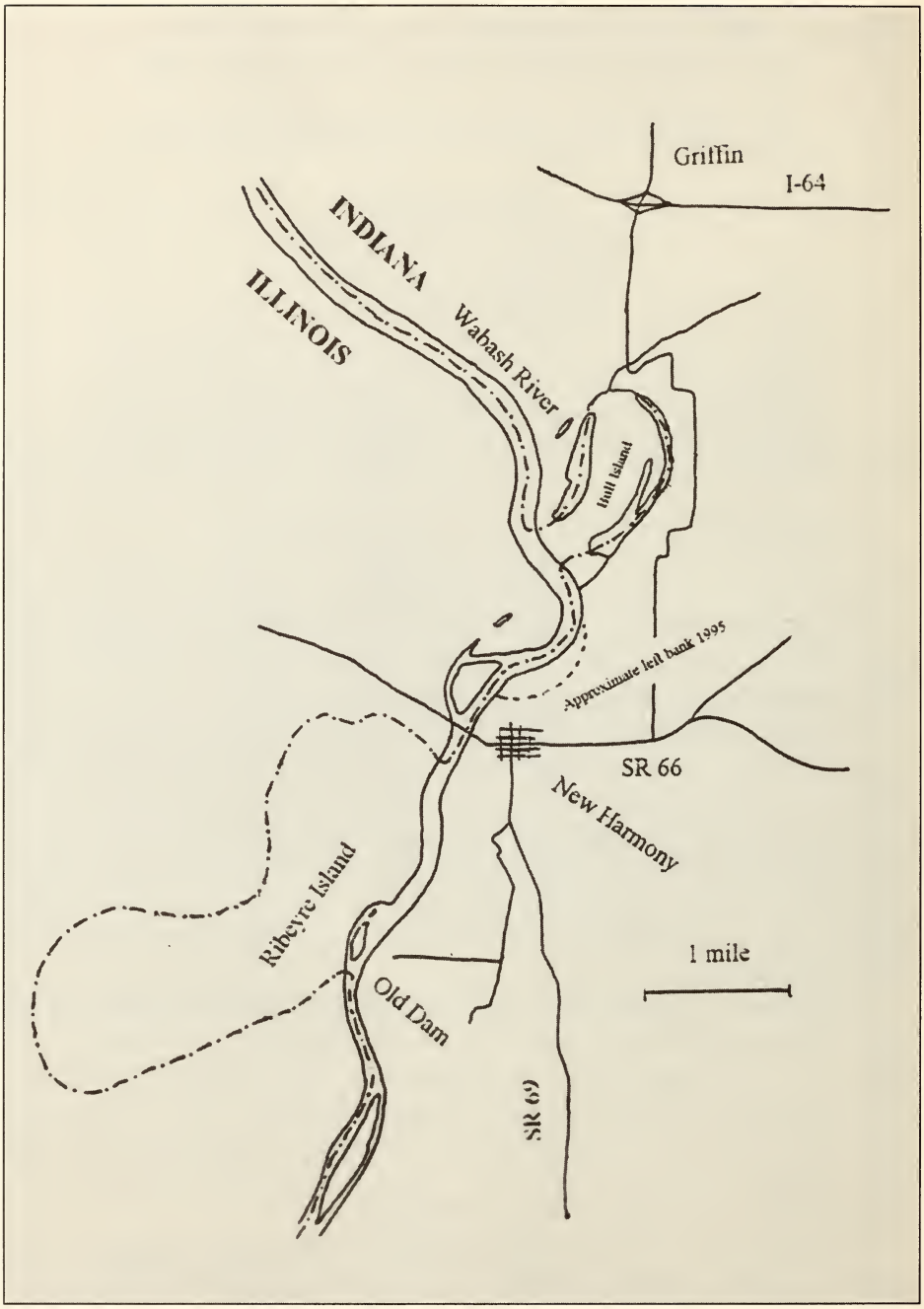

Figure 1. New Harmony and vicinity.

flooding along many of its tributaries, resulting in the formation of slack-water deposits. The town of Poseyville lies on such an old lake bed. The flat topography and the black, rich soils provide mute testimony to past glacial activity.

The Wabash River was the temporary outlet of the Great Lakes as long as ice blocked the present-day St. Lawrence River. When the water from glacial Lake Maumee (Lake Erie) broke through the moraine near Fort Wayne, the flooding that resulted along the Wabash River was instrumental in the development of the Maumee Terrace. This is the same terrace system upon which the Harmonist settlers from Pennsylvania founded their town of Harmonie, today's New Harmony.

WABASH RIVER

The Wabash River continues to have a major influence on Posey County, its natural resources, and the development of those resources. The first accounts of the utilization of the natural resources of the New Harmony area were sketchy references to the taming and channeling of the Wabash River by the Harmonists. George Rapp and his Millenialist followers established Harmonie in 1814. To operate their gristmill, they channeled the river through the bayou southward for about a mile to a place known today as "The Old Dam." Here, the river flowed into the area from the west and made a right angle bend to the south. By locating their grist mill here, the Harmonists took advantage of a bedrock ridge which extends to the west and is formed by a series of Pennsylvanian-age channel sands or sediment-filled river channels. This accident of bedrock topography causes the Wabash River to drop several feet in elevation across the channel sands, providing a corresponding head of water that was used to operate the mill.

Man-made changes to the Wabash River over the next two hundred years profoundly affected the town of New Harmony and the Wabash River itself. The Harmonists had established a thriving economic enterprise in Pennsylvania, and their move to the banks of the Wabash necessitated continuing contact back East. In the early days of the settlement of Harmonie, the river was the only major transportation and communications route. Overland transportation was, for all practical purposes, non-existent, and sending goods either to the East or South involved the use of the Ohio and/or Mississippi Rivers. The waterway made trade possible because river transport could move bulky materials, such as limestone for buildings and coal for fuel. The Harmonists and the community they founded depended on trade; they were more than simply a group of Millenialists awaiting the second coming of Christ. Their business activities provided a surplus for trade. For example, the Harmonists grew fibers suitable for making rope. The old ropewalk, where raw rope dried in lengths before being shipped, stretches across one end of the town and is a major tourist attraction in New Harmony. By one account, in 1824, Harmonist commerce reached 22 states and ten countries. In 1820, the value of the goods manufactured in Harmonie reached $50,000. As a result, Posey County exceeded all but one other Indiana county in income from trade (Pitzer and Elliott, 1979).

EROSION AMD MEANDERS

The Wabash River, especially from Vincennes to its confluence with the Ohio River, has a meandering course. The river moves through a series of loops that tend to enlarge, cut themselves off, and then re-establish themselves as the river flows south. This serpent-like waterway moves across its floodplain as the course of the river changes. Any obstruction in one part of the channel leads to course changes elsewhere over time. The entire floodplain is marked with old meander scars. Since the town of New Harmony is located on a terrace, the site is not subject to flooding, unless the water level in the Wabash is extremely high. The gradual shift of the main channel to the bayou of the Harmonist cut-off eventually blocked navigation on the Wabash River upstream from the "Old Dam" location and formed modern Ribeyre Island.

In January 1826, the keelboat *Philanthropist* (called the "Boatload of Knowledge" because of its cargo of utopian educators, part of Robert Owen's social experiment in communal living) arrived in New Harmony. The boat came upriver from the Ohio River, and the passengers disembarked at the town. Today, the rapids and low water across the "Old Dam" make the Wabash River unnavigable here except during periods of extreme flooding, and then the rapids are hazardous. River traffic from the Ohio River to New Harmony essentially became impossible as a result of the rechanneling of the Wabash River. The opening of the watercourse across the bayou in 1814 made it possible for the Wabash River, during periods of high water, to widen this new course at the expense of the old channel. The final shift in the channel occurred after 1826, but the exact date is unknown. A sketch dated 1834 and attributed to Charles-Alexander Lesueur shows a significant flow in the original "bayou" (Carmony and Elliott, 1980).

The U.S. Geological Survey map of 1903 (the New Harmony, Indiana-Illinois quadrangle) shows the main channel extending around modern Ribeyre Island and a "cut off" where the original bayou was located. The U.S. Geological Survey quadrangle map of 1959 shows that the channel had completely changed.

The changing meanders also profoundly affected the area at another location. In 1920, the meander forming the loop around Bull Island, just upstream from New Harmony, was cut off, leading to the formation of a meander loop just north of the town itself. This meander has been eroding the Maumee Terrace north of New Harmony at the rate of 50 feet per year (U.S. Army Corps of Engineers, 1992; Shaver, 1979), and, if left unchecked, the town itself will be threatened by the year 2000. A permeable jetty system was built in 1986, but the average rate of bank erosion continued essentially unchanged after its construction. The U.S. Army Corps of Engineers and representatives from the town itself are aware of the problem and are working to ameliorate the danger.

TRANSPORTATION ENGINEERING

Overland transportation to and from New Harmony developed slowly. Trails were improved as southwestern Indiana was settled. These trails included the

overland trail to Mount Vernon, the old Buffalo Trace from Vincennes to Louisville, and the Red Bank Trail to Evansville and Henderson, Kentucky. These early roads were not user-friendly, to use a modern term. A description of the day-long, overland trek from Mount Vernon to New Harmony in January 1825 is instructive as it tells of a rough road and slow-moving wagons. Mud gummed up the wagon wheels and affected horses and people adversely (Wilson, 1964). A major engineering feat was celebrated when this road was improved by using a plank surface (*i.e.*, became a corduroy road), a technique known since the Middle Ages (Schia, 1991). Kelley (1951) provides a revealing description of the construction of this road. She writes that the road was graded to a width of 18 feet with essentially two lanes, one of plank and one of soil. The construction must have started in the mid-1840's and was completed in 1851. The planks that covered the road were eight feet in length and two inches thick. Their width varied from four to 18 inches. A variation of the same road-building method is occasionally used today when a road is constructed across soft soils. Logs are felled across the roadway to form a corduroy foundation for the road surface. As recently as the construction of the trans-Alaska oil pipeline in the 1970's, a similar technique was used to preserve the permafrost environment by careful hand clearing and preservation of the vegetation mat before adding base materials for access roads or the pipeline work pad itself.

Kelley (1951) also discussed improvements to the roads in Posey County over time. She credits John B. Elliott as the driving force behind the improvement of the county roads to all-weather or gravel roads. This change may have occurred as early as the 1870's (Josephine M. Elliott, pers. comm., 1996), predating the "Good Road" movement by at least 50 years. John B.'s son, John S. Elliott, would eventually obtain a degree in Civil Engineering from Purdue University and become the engineer in charge of construction of the current steel truss bridge which crosses the Wabash River at New Harmony.

LOCAL RESOURCES

When the Harmonists first founded their town of Harmonie, they literally carved a community out of the wilderness. The only reliable link with the outside world was the river. In a matter of months, they cut lumber for buildings and used local clay to make bricks. From outcrops along the Wabash and Ohio Rivers, they shipped blocks of limestone and sandstone for buildings. An early map of New Harmony shows the town clay pit just to the south of the original settlement. A description of the bricks and their production (Pitzer and Elliott, 1979, p. 269) includes a reference to some writing on one brick in German: "Harmonie der 25 Mai 1823 ist ein sehr schoner Tag wir haben 2400 Bachstein gemacht. Morgenstund hat Gold im Mund." (Harmonie 25 May 1823 is a very beautiful day we have made 2400 bricks. Morning hour has gold in [the] mouth.) The last portion is a Germanic rime phrase (known not only in Germany but also in Scandinavia) that is roughly equivalent to "the early bird gets the worm."

Iron tools must have been imported because no evidence exists indicating that iron was made from local ores. Bog iron was mined in northern Indiana, and the coal near Terre Haute formed the basis for iron making in central Indiana (Wayne, 1970).

Coal for fuel was probably exploited very early in Posey County. Numerous thin coal seams lie exposed in the area, and excavation would pose no problems. The coal could be loaded on a boat and transported to New Harmony or elsewhere.

The source of water power has already been described. The Harmonists operated their grist mill from the very beginning of the settlement. When Robert Owen bought the community for his social experiment, he bought a fully functional town, including cleared fields, beasts of burden to plow and transport, animals for food and wool, and sturdy structures for a variety of purposes. The land itself was also a valuable resource.

About 80% of the County is actively farmed. The main crops are corn, wheat, and soybeans (U.S. Department of Agriculture, 1979). The County also possesses two recreational areas: Hovey Lake and Harmonie State Park. The surface soils are placed in three broad categories (Yeh, 1982): eolian deposits, fluvial deposits, and marsh and swamp lands. Windblown sand deposits occur along the bluff of the Wabash River. Windblown silt or loess covers all of the upland. More than 60% of Posey County is covered by fluvially deposited materials. The soils make good farmland, whose main problem is drainage, necessitating the use of ditches or field tiles. The combination of soft, silty topsoils, poor drainage, and frost action causes the roadbed break-up and stability problems that test the ingenuity of today's road builders.

INTELLECTUAL RESOURCES

Owen and his followers are best remembered for their utopian community and intellectual pursuits. New Harmony was essentially an outpost for intelligentsia. At the time, the western frontier was the edge of the eastern forest. The western prairies started just beyond New Harmony and the Wabash River in eastern Illinois. Connecting routes to the east and south were along the rivers.

Owen's social experiment, according to some of his critics (Wilson, 1964), had one major flaw—it was top heavy. The emphasis was on intellect and not on people who would be actual producers, laborers, farmhands, and others with similar pursuits. A brilliant mind does not assure that the crops are planted and harvested on time or that the pigs are fed. Despite this, a tremendous surge in resource development occurred in the community of New Harmony. The new nation was in the midst of its westward and northward expansion. The Ohio River was a finger pointing into the wilderness; at the end of the pointer was a mother lode, an enormous intellectual resource unparalleled elsewhere on the western frontier, the people of New Harmony. Robert Owen's "Boatload of Knowledge" brought together a group of scientists and naturalists who would influence resource development on the new frontier. A world class geologist, William Maclure, was

in the company of great scientists like Thomas Say and Charles-Alexandre Lesueur. Two of Robert Owen's sons would grow up to be naturalists casting long shadows.

William Maclure was Robert Owen's partner in the New Harmony experiment. At that time, Maclure's reputation as a geologist and educator was firmly established. His map of the geology of the eastern part of the continent and his lecture in 1809 to the American Philosophical Society established him as the "Father of American Geology." Maclure was a member of the Academy of Natural Sciences of Philadelphia and served as its President from 1817 until his death in 1840 (Pitzer, 1989).

Maclure was instrumental in attracting other natural scientists and educators to Owen's social experiment. He convinced the former President of the Philadelphia Academy, Gerard Troost, to come to New Harmony. Troost, a Dutch geologist and mineralogist, would later be a Professor of Geology in Nashville and the State Geologist of Tennessee. Troost spent the years between 1825 and 1827 in New Harmony. However, he did not arrive on the *Philanthropist* but arrived a month earlier in New Harmony by traveling overland (Wilson, 1964). Thomas Say, Professor of Natural History at the University of Pennsylvania and the "Father of American Entomology," was another of Maclure's friends from the Philadelphia Academy. Say accompanied Maclure on the boat to New Harmony. Lesueur was the third member of Maclure's group of scientists. He was a French naturalist and artist, whom Maclure had first met in Paris. He would later accompany Maclure on geologic expeditions to the West Indies and elsewhere in the United States prior to assuming the duties of Curator for the Philadelphia Academy.

The scientists of New Harmony were visited by many of the outstanding minds of the times, both from within the United States and also from Europe. Sir Charles Lyell, one of the founders of modern geology, and Lady Lyell visited in 1846. Lyell's interpretation and dissemination of the concept of uniformitarianism was trail blazing, and the concept is still one of the cornerstones of modern geology (Tarbuck, 1994).

Prince Maximilian of Wied spent the winter of 1832-1833 in residence. The Prince, himself a naturalist, commented in his diary on the flora and fauna and described hunting trips in the general area. He spent considerable time with Say and Lesueur studying local collections, and he spoke highly of their quality. The Prince also made a few remarks regarding the natural resources of the County. He described the muddy roads but also commented on the fertile, black soils. The record of his trip, *Travels in the Interior of North America, 1832-34*, was published in Koblenz between 1838 and 1840. Translations into French and English soon followed. (Notes from an annotated, unpublished translation of the manuscript were generously provided by Dr. Josephine M. Elliott of Historic New Harmony.)

David Dale Owen arrived in New Harmony with his brother Richard in 1828. David Dale went to Cincinnati to obtain a medical degree, not in order to prac-

tice medicine, but to receive a solid foundation in the sciences. During the summer of 1836, David Dale Owen served as an assistant to Troost, then the State Geologist of Tennessee, and in 1837, after completing his degree, he was appointed geologist for the State of Indiana. He traversed the State over the next year and a half. Owen's report is an extremely impressive document (Owen, 1837). The report established the stratigraphy of the State, gave the approximate geologic age of the rock formations, and described the major geologic resources of Indiana — coal, limestone, and sandstone. Owen also correlated the rock formations of Indiana with those in Europe of similar age and lithology, extending stratigraphic correlations across the Atlantic Ocean. He was one of the first geologists to accomplish this. His report failed to mention only two of the resources that have contributed to Indiana's economy since statehood — petroleum and gypsum. The Drake well in Pennsylvania was not drilled until more than 20 years after Owen's original work. Gypsum, since the mineral is not exposed in outcrop, was not discovered until the 1950's.

In 1839, David Dale accepted a federal appointment to survey the mineral lands of the United States, leading to investigations of the lands to the north and west of Indiana. His report (Owen, 1852) to the Commissioner of the General Land Office in Washington, D.C., is a massive document. The introduction presents a concise summary of his geologic findings as well as practical advice on the logistics of performing geological surveys across large areas with essentially no infrastructure. His principal assistant was another New Harmony resident, Joseph Granville Norwood, who would later become State Geologist of Illinois (Kimberling, 1966). The town of New Harmony, Indiana, became the headquarters for federal geological surveys. During this time, Owen also remodeled the old Harmonist Granary, the "Old Fort," which housed the Maclure collection and literally tons of material from his own trips. When Sir Charles Lyell visited New Harmony in 1846, he examined the collections and, with Owen, visited points of interest in the local area (Shaver, 1987).

In 1854, Owen was appointed State Geologist of Kentucky and, in 1857, State Geologist of Arkansas. In 1859, Indiana again appointed him State Geologist, but due to ill health, he accepted with the proviso that his brother, Richard Owen, would start the work of the new survey. Following David Dale's death in 1860, the second Indiana Survey was completed by Richard. This survey included a detailed investigation of the coal and sandstone resources of the State.

The Civil War interrupted the work of the geological survey. Richard Owen emerged from the war as a colonel and resumed his scientific work, which led to a life-long affiliation with geology and Indiana University. He served briefly as the first President of Purdue University, and, in 1885, he became one of the charter members of the Indiana Academy of Science — only three of the milestones in the career of a geologist from a small town in southwestern Indiana.

The geological tradition continued at New Harmony after Richard Owen's departure. In 1869, Edward Travers Cox of New Harmony and a former asso-

ciate of Richard Owen was appointed State Geologist of Indiana. His 1875 report (Cox, 1876) contains a brief reference to Posey County in the analysis of an 18-inch coal seam ("George Heldferl's coal") near St. Wendel. In 1879, his assistant, John Collett, succeeded him as State Geologist. The beacon of the frontier illuminated the way for resource development, not only in a County in southwestern Indiana but for the entire State as well as much of the Midwest and South, ranging from Arkansas, Illinois, and Iowa to Michigan, Wisconsin, and Minnesota. During the period from 1830 to 1860, geologists from New Harmony accounted for more survey participants than from any other group, a total of 14 of 56 projects or one quarter of the total (Johnson, 1977). Quite impressive for a small town without a university.

THE LAST CHAPTER

As mentioned previously, David Dale Owen did not include oil and gas in his survey report of 1837-1838. This chapter in the development of Posey County was not written for a long time. The Wabash Valley Fault System guarded information unknown to the early geologists. Although oil seeps had been reported in Indiana, a big strike did not occur until 1886 when the Trenton Gas Field was discovered in central Indiana. Oil was discovered in the Illinois Basin the same year. The Illinois Basin is a large structural basin centered in Illinois that covers about 60,000 square miles. Posey County and the Wabash Valley Fault System are on the eastern edge of the Illinois Basin. The oil is found in the sandstones and limestones of the basin, and the source rock is probably the black organic shales that occur throughout the formation. Oil was discovered in the Indiana portion of the Illinois Basin in 1889 when a well was drilled in Terre Haute. Small discoveries were made throughout southwestern Indiana from 1912 to 1938, but the oil traps were relatively small and difficult to locate from the surface. This difficulty was overcome with the advent of geophysical exploration techniques that enabled geologists to evaluate formations at depth. In 1938, a discovery was made near Griffin that led to the development of the oil industry in southwestern Indiana. As the faults are an integral part of the oil traps, the Wabash Valley Fault System is outlined by the oil wells that stretch, much like pearls on a string, up and down the fault trace.

An interesting footnote exists to the oil story. The Harmonists, having returned to Pennsylvania after selling Harmonie to Robert Owen, had a share in the early development of the petroleum industry in that State.

SUMMARY

New Harmony, Indiana, a living, historical community, was the location of two communal social experiments. Although Robert Owen's secular experiment was short-lived (1824-1826), the scientists he gathered and their followers left a permanent stamp on resource development across Indiana and in the central portion of the United States. The area experienced a resurgence during the pre-World War II oil boom. Today, New Harmony is a major tourist attraction, draw-

ing people from all over the world. In its academic depth and its historic restoration, the site proudly displays its rich heritage of resource development on the frontier.

ACKNOWLEDGMENTS

The field and library work for this project were funded by the Historic New Harmony organization of the University of Southern Indiana, whose generosity is gratefully acknowledged. The support of Drs. Josephine and John Elliott of Historic New Harmony with their detailed knowledge of unpublished manuscripts and oral history is gratefully acknowledged, as is the support of Dr. Jane Thompson Johansen for editorial help and her insight into the town of New Harmony.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 267-290

THE VASCULAR FLORA OF FOGWELL FOREST NATURE PRESERVE, ALLEN COUNTY, INDIANA

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ABSTRACT: This flora of Fogwell Forest Nature Preserve (Allen County, Indiana) documents 243 vascular plant species within the 28 acre site. Approximately 60 species, including *Stachys cordata*, are reported for the first time for Allen County. The physical and biotic history of the site are reviewed, and the major community types, especially the flat hardwood community, are described. Although few threatened or endangered species are included within the flatwoods, the virtual lack of exotic or weedy species indicates that Fogwell Forest has good preserve quality. This conclusion is further supported by an average coefficient of conservatism of at least 5.6 and a high floristic quality index (> 80).

KEYWORDS: Allen County — flora, county records — vascular plants, flatwoods, flora — Indiana, swamps — Indiana, *Stachys cordata*.

INTRODUCTION

The Central Till Plain Natural Region (Homoya, *et al.,* 1985) represents a major vegetation and physiographic zone within Indiana. This glacial till formation extends from south of Indianapolis to Fort Wayne in the northeast and from the Ohio State Line to the Wabash River Valley in the west. Historically, the gentle topographic relief and highly tillable soils have resulted in the clearing of most of the land for agricultural uses. In Wabash County, typical of the Till Plain Natural Region, less than 5% of the County currently is forested (Hicks and Keller, 1996). Furthermore, the remnant forest might now be visualized as a series of biological islands since the average woodlot seldom exceeds 5 acres in extent and is surrounded by cultivated fields, old fields, pasture, and human habitation. Given this high degree of disturbance and fragmentation, it is not surprising that the region possesses so few dedicated nature preserves. Of the approximately 120 Indiana Nature Preserves (Indiana Department of Natural Resources, 1991), only 14% are found in the Central Till Plain Natural Region, and the majority of these are clustered near the cities of Indianapolis and Fort Wayne.

Fogwell Forest Nature Preserve, a 28 acre site owned and managed by Acres Land Trust, Fort Wayne, Indiana, contains an excellent example of a mature Central Till Plain second-growth flatwood forest. Since becoming a preserve in 1976, only informal study of its flora has been undertaken. The following report seeks to provide a full, baseline inventory of the vascular flora of the area, with documented voucher specimens, and to describe recent physical and historic factors which have shaped its flora.

MATERIALS AND METHODS

In order to fully document the flora, the Preserve was visited intensively during the 1993 growing season — twice monthly in May and June, and monthly through the remainder of the season. Since the Preserve is fairly small and without a well-defined system of trails, sampling was done by wandering through the Preserve. The path varied with each visit. Occasional follow-up surveys were undertaken during 1994 and 1996 to insure that, to the extent possible, no species had been missed. Voucher specimens of each species observed were collected and deposited in the Friesner Herbarium at Butler University (BUT). Nomenclature follows Gleason and Cronquist (1991). Species were deemed unreported for Allen County if they did not appear in the computer database of Keller, *et al.* (1984).

The floristic quality of the wooded portion of the Preserve was assessed by two quantitative measures, namely the mean coefficient of conservatism (C) and the floristic quality index (I), both devised by Swink and Wilhelm (1994). Swink and Wilhelm assigned C values (ranging from 0-10) to native species based upon their fidelity to specific microhabitats. The mean of C ($\bar{C}$) is determined from the number of native species present at the site being analyzed. The floristic quality index (I) is equal to $\bar{C}\sqrt{N}$, where N equals the number of native species in the study area. In contrast to $\bar{C}$, the floristic quality index provides an assessment of native species diversity, which theoretically should be high in intact natural communities.

DESCRIPTION OF THE AREA

Fogwell Forest Nature Preserve, located in the northwest $^1/_4$ of Section 1 and an adjacent portion of Section 39 (Banstetters Reserve), T29N, R11E (41° 0′ N, 85° 14.5′ W), contains about 28 acres of old second-growth forest with woodland vernal pools. The Preserve is surrounded by agricultural land and a housing subdivision. Acreage northwest and southwest of the forest block is still used for growing row crops. Acreage to the south and east has been out of production since 1993 when an unsuccessful attempt was made to utilize this tract as a wetland mitigation site. In the mitigation effort, agricultural drainage tiling was disabled and a few species of woody and herbaceous plants were installed. Since 1993, natural ecological succession has been allowed to reclaim this tract. To some extent, agricultural tiling has modified the hydrology of Fogwell Forest. Glenn Fogwell recalls one length of tile extending about 150 feet into the eastern portion of the woods to drain a small pond. This tiling may still be functional since, during one high water period, water was observed to flow over ground and into a drain hole (Rich Dunbar, pers. comm.).

Fogwell Forest lies just within the Mississippi River (as opposed to the Lake Erie) watershed in southwestern Allen County. Specifically, the forest sits on glacial till of the Wabash Moraine (Kirschner and Zachary, 1969) or the adjacent ground-moraine (Bleuer and Moore, 1978). This geologically young deposit,

over 100 feet thick, was left by the retreat of the Erie Lobe of the Wisconsinan glacier approximately 12,000 years ago (Bleuer and Moore, 1978).

The resulting soils, belonging to the Blount-Pewamo Association, are Blount silt loams (Kirschner and Zachary, 1969). This soil type is deep but somewhat poorly drained. The 9-inch A horizon is friable, dark grayish-brown, and slightly acidic. Subsoils are friable to firm, mostly with distinct mottling (indicating a non-oxidizing environment), and strongly acidic. The underlying calcareous parent material ranges from 20 to 40 inches below the surface.

A second nature preserve, Fox Island (Lindsey, *et al.*, 1969), is located within 1.5 miles of Fogwell Forest Nature Preserve. With about 200 acres, Fox Island is much larger and more diverse in habitat and flora than Fogwell Forest (Oliphant, 1988); it includes a cattail marsh and some open water. Furthermore, being associated with a morainal dune, much of Fox Island has sandy substrate covered with young upland forest dominated by *Quercus velutina*. In contrast to Fox Island, Fogwell Forest offers an unusually fine example of flatwood vegetation. The site exhibits more or less flat topography and poorly drained depressions. Very slight elevational changes modify the forest vegetation from vernal pool with *Quercus bicolor* (and occasionally, *Q. macrocarpus*) to mesic woods with *Acer saccharum*, *Fagus grandifolia*, *Q. alba*, and *Q. rubra*.

THE PLANT COMMUNITIES

Historically, the combination of geology, soils, and climate that prevailed across Indiana's Central Till Plain Natural Region gave rise to a regional forest dominated by *Fagus grandifolia* and *Acer saccharum*. Homoya (1985) considers Fogwell Forest, in particular, to be at the northern edge of the Bluffton Till Plain Section, a transition zone between the forests of northern versus southern affinities. Before dissection of this forest for agriculture and settlements, the Bluffton Till Plain had, in addition to forests of *F. grandifolia* and *A. saccharum*, an abundance of flatwoods with hydrophytic woody species, such as *Acer rubrum*, *Quercus bicolor*, and *Fraxinus pennsylvanica*. The Fogwell Preserve exemplifies this combination of meso- and hydrophytic forest communities.

Before being donated to Acres Land Trust by Glenn Fogwell and his sisters Mabel Corville and Mildred Klopfenstein in 1976, the land had been part of the family farm for 3 generations. According to Glenn Fogwell, some trees were removed from the western part of the present-day preserve by his grandfather. During the 1930's, following several visits by State Forester Charles Deam, the tract was designated a classified forest. This status allowed the property owners to sell or remove old trees but required them to leave new growth and to never clear the ground. As a result, an unspecified number of trees down to one foot in diameter were sold from the eastern 13 acres of the forested area. Between the 1930's and 1976, no further live trees were felled, but dead wood was removed for firewood. During the known history of this tract, no selective disturbance of the herbaceous vegetation, such as collecting medicinals or the removal of plants for sale or use elsewhere, ever occurred. The logging of the 1930's undoubt-

edly had some effects on the herbaceous layer, but, at the time of this survey, no apparent differences in species richness were noticed between the western and eastern portions of the Preserve. Today, the vegetation of Fogwell Forest Nature Preserve may be visualized as having 3 distinct community types: flatwoods, ecotone, and adjacent old field.

Flatwoods. The core of the Preserve is the 28 acre flatwoods dominated by mixed hardwoods. Associated with vernal pools are mature individuals of *Quercus bicolor* and a sparse herbaceous flora that includes *Carex bromoides*, *C. grayi*, and *C. muskingumensis* and the ferns *Onoclea sensibilis, Athyrium* sp., *Dryopteris* sp., and *Thelypteris* sp.

In the more mesic areas, the canopy layer is dominated by *Acer saccharum*, *Fagus americana*, and *Quercus rubra*. Subdominants of the canopy include *Juglans nigra*, *Carya ovata*, *Quercus alba*, and *Tilia americana*. During the study, several mature individuals of *F. americana* succumbed. At the same time, no loss of other dominant canopy trees was observed. Given that the forest contains some younger and sapling stage *F. americana*, these losses may not have a long-term effect on canopy composition.

The mesic understory layer contains *Lindera benzoin*, *Viburnum prunifolium,* and *Aesculus glabra* as well as scattered areas with *Carpinus caroliniana* and *Ostrya virginiana*. *Ulmus rubra* grows in openings and near the edge of the woods. Although numerous, *A. glabra*, *C. caroliniana*, and *U. rubra* failed to produce seed crops during the observation period.

The herbaceous layer proved to be particularly rich in the spring and early summer. The earliest cohort to flower includes drifts of *Trillium grandiflorum* and abundant *Cardamine concatenata*, *Geranium maculatum*, *Osmorhiza longistylis*, *Asarum canadense*, *Arisaema triphyllum*, *Phlox divaricata*, and *Galium aparine*. Three species of *Hydrophyllum*, but especially *H. macrophyllum*, were also common. Although *Carex* species are diverse, none proved to be dominant within the herbaceous layer. *Carex woodii*, due to its rhizomatous habit, occupies some significant area in the western portion of the woods.

The early summer dominants of the herbaceous layer include *Laportea canadensis* as well as *Cryptotaenia canadensis*, *Sanicula gregaria*, and *Smilax ecirrhata*. *Parthenocissus quinquefolia* is the dominant vine species in the Preserve. Common grass species include *Elymus hystrix* and *Festuca subverticillata* as well as *Cinna arundinacea*.

In later summer, significant flowering populations of *Impatiens capensis*, *Collinsonia canadensis*, and *Polygonum virginianum* were observed. In contrast, populations of autumn-blooming species were small and only attained meaningful size near the margins of the forest. The most frequent autumn-blooming species include *Aster cordifolius*, *A. lateriflorus*, *Solidago caesia*, and *S. flexicaulis*.

Ecotone Between Woods and Fields. The edges of the woodland provide suitable but limited habitat for sun-loving woody and successional species. The tree species *Quercus muhlenbergii* and *Prunus serotina* occupy the ecotone

area but were not observed within the Forest Preserve itself. Likewise, the vine *Toxicodendron radicans* is virtually restricted to this ecotone. Common woody species at the woodland edges include *Rubus* spp., *Viburnum prunifolium*, *Rosa setigera*, and *Crataegus* spp. Among herbaceous species, the most abundant are *Achillea millefolium*, *Aster pilosus*, *Helianthus tuberosus*, *Solidago canadensis*, and *Trifolium* spp.

Old Field. During the observation period, the adjacent old fields were in a pre-*Solidago* stage. The grasses *Bromus inermis*, *Dactylis glomerata*, *Festuca elatior*, and *Poa pratensis* were common as were the dicot perennials *Plantago* spp., *Trifolium* spp., and *Fragaria virginiana*.

FLORISTIC NOTES

Within the Preserve, no members of the family Orchidaceae were noted. Their absence contrasts sharply with the four species found in the larger, more diverse Fox Island Nature Preserve (Oliphant, 1988). On the other hand, the quality and richness of the Fogwell site are supported by other observations. The Preserve as a whole supports 243 species, including 10 fern species and 1 fern ally. Species in the flatwoods, including its edge species, had a mean coefficient of conservatism ($\bar{C}$) of 5.6 (*i.e.*, an above average value). The mean coefficient increased to 6.3 when the edge species were deleted from the analysis. Most impressive, however, is the floristic quality index (I) of 81.4. According to Swink and Wilhelm (1994), a remnant community with an I value greater than 45 has natural area potential. In fact, in their study area of northwestern Indiana, northeastern Illinois, and adjacent Wisconsin, communities scoring 50 or higher were extremely rare.

Of further value in assessing the floristic quality of Fogwell Preserve, the core flatwoods is remarkably free of exotic and weedy species. *Rosa multiflora*, for example, does not extend beyond the forest margin. Most exotic species, such as *Setaria faberi*, are limited to well-lit sites, such as the old field. With the exception of *Lonicera morrowii*, currently growing in the ecotonal area, the exotic species observed are likely to quickly diminish with successional maturation of the old fields.

Among the species verified in this flora, 61 are new for Allen County (*cf.* Keller, *et al.*, 1984). Although about a third of these might be considered weedy or exotic species, the majority are woodland native species. This number is a surprisingly large for what may be thought of as a well-collected region. Two explanations might be offered. First, much of the "botanizing" of the region was informal *(i.e.*, visual reports were made, but no voucher specimens were collected). This is certainly true of the nearby Fox Island Nature Preserve (Ronald Zartman, Fox Island Naturalist, pers. comm.). Second, the area was undercollected by Charles Deam while he was preparing his *Flora of Indiana*. One might speculate that Deam tended to ignore Allen County in favor of his home area of Wells County as well as the botanically richer counties to the north.

Although Deam rarely collected in Allen County, his visits to the Fogwell property are easily documented. Some visits may have been solely in an official capacity leading to designating the area as classified forest. On the other hand, Deam did collect specimens from the site on 9 June 1933; his total for the day consisted of 30 specimens, half of them from the Fogwell farm (Lewis Johnson, pers. comm.). Deam's accession notes indicate that some were from "open space," while others came from a "low spot." At least 2 species collected by Deam are not found within the present-day Preserve—*Carex tenera* var. *echinodes* and *Ranunculus recurvatum*. The first taxon is of particular interest since it has a limited global distribution. Many areas of potentially suitable habitat for *Carex tenera* var. *echinodes* lie outside the Preserve in the extended backyards of nearby housing; the species should still be sought there.

Among the non-weedy species not previously documented for Allen County, *Stachys cordata* is of greatest interest. Homoya (pers. comm.) describes this taxon, characterized by long petioles and stipitate-glandular stems, as mostly from the southeastern and south-central portion of the State though the species is not as rare as indicated by Deam (1940). This species has not been reported for nearby Michigan (Voss, 1996) but does apparently occur in western Ohio in Auglaize and Shelby Counties (Cooperrider, 1995). Gleason and Cronquist (1991) characterize *S. cordata* as chiefly Appalachian in distribution. Thus, Allen County must be at or near the northern and western limit for this species. Oliphant (1988) lists (but does not document) a plant, tentatively identified as *Stachys nutallii*, for nearby Fox Island Nature Preserve. Given that this is a taxonomically and nomenclaturally confusing entity, Oliphant's specimen very likely represents another occurrence of *S. cordata* in Allen County.

Another species documented for Fogwell Forest, *Carex woodii*, was considered by Deam (1940) to be a rare plant of northern Indiana. Not surprisingly, Bacone and Hedge (1980) originally placed this species on their state endangered and threatened plant list. However, previous floristic studies often overlooked *C. woodii*, perhaps by confusing the species with clones of *C. pensylvanica* or because of sparse flowering and fruiting. *Carex woodii* has been seen widely in rich woods throughout northeastern Indiana.

ACKNOWLEDGMENTS

My thanks to Acres Land Trust, Rebecca Dolan (Friesner Herbarium, Butler University) for curatorial assistance, Lewis Johnson (Indiana University Herbarium) for special insights into the work of Charles Deam, and Rich Dunbar and Ronald Zartman for information on the flora of Fox Island Nature Preserve. Also, my appreciation to the anonymous reviewers for their careful reading of the manuscript which improved the final product.

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CATALOG OF VASCULAR PLANTS IN FOGWELL FOREST NATURE PRESERVE ARRANGED ALPHABETICALLY BY FAMILY WITHIN DIVISION[1]

DIVISION EQUISETOPHYTA — Scouring Rushes

Equisetaceae — Horsetail Family

Equisetum arvense L.
Field horsetail; rare in woods; collection number = 2796; coefficient of conservatism[2] = 0.

DIVISION POLYPODIOPHYTA — Ferns

Adiantaceae — Maidenhair Fern Family

Adiantum pedatum L.
Northern maidenhair fern; infrequent in mesic woods; 3025; 10.

Aspleniaceae — Spleenwort Family

Athyrium filix-femina (L.) Roth [*A. angustum*]
Lady fern; infrequent in damp woods; 3386; 8.

\+ *Athyrium thelypterioides* (Michx.) Desv.
Silvery glade-fern; frequent in damp woods; 3060B; 10.

\+ *Cystopteris protrusa* (Weatherby) Blasdell [*C. fragilis* var. *protrusa*]
Lowland bladder-fern; frequent in woods; 2966; 6.

Dryopteris carthusiana (Villars) Fuchs [*D. spinulosa*]
Toothed wood-fern; infrequent in damp woods; 3132; 6.

\+ *Dryopteris goldiana* (Hook.) Gray
Goldie's wood-fern; infrequent in damp woods; 3133; 10.

\+ *Thelypteris hexagonoptera* (Michx.) Weatherby [*Dryopteris hexagonoptera*]
Broad beech-fern; occasional in damp woods; 3384; 10.

Onocleaceae — Sensitive Fern Family

Onoclea sensibilis L.
Sensitive fern; infrequent in wet woods; 3064; 8.

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[1] + = Not previously reported for Allen County (based upon C. Keller, *et al.*, 1984). The scientific names in brackets are synonyms used in Deam (1940).

[2] Non-native species are not given a coefficient of conservatism (C) nor are they used to calculate the floristic quality index (I) (Swink and Wilhelm, 1994). The coefficient of conservatism is also not listed for native species collected from fallow or old fields; these species (marked na = not applicable) occur outside the core Preserve and were not included in the calculation of I.

Ophioglossaceae — Adder's Tongue Family

Botrychium dissectum Spreng. var. *obliquum* (Muhl.) Clute
Lace-frond grape-fern; rare in woods; 3134; 6.

Botrychium virginianum (L.) Swartz
Rattlesnake fern; frequent in mesic woods; 3027; 6.

DIVISION MAGNOLIOPHYTA — Flowering Plants

Aceraceae — Maple Family

Acer rubrum L.
Red maple; infrequent in moist, wooded openings; 3291; 7.

+ *Acer saccharum* Marsh
Sugar maple; abundant in mesic woods; 3189; 3.

Anacardiaceae — Cashew Family

+ *Toxicodendron radicans* (L.) Kuntze var. *negundo* (Gr.) Reveal [*Rhus radicans*]
Common poison-ivy; frequent at wood edges; 3083; 2.

Annonaceae — Custard-Apple Family

Asimina triloba (L.) Dunal
Papaw; occasional in woods; 3059; 9.

Apiaceae — Carrot Family

Cryptotaenia canadensis (L.) DC.
Honewort; common in mesic woods; 3055; 2.

+ *Daucus carota* L.
Wild carrot; edge of fields; 3145; non-native.

Erigenia bulbosa (Michx.) Nutt.
Harbinger of spring; frequent in mesic woods; 2785; 10.

Osmorhiza longistylis (Torrey) DC.
Long-styled sweet cicely; abundant in woods; 2843, 2967; 3.

+ *Sanicula gregaria* Bickn.
Cluster sanicle; common in woods; 3056; 2.

Sanicula trifoliata Bickn.
Beaked sanicle; infrequent in woods; 3072; 10.

Apocynaceae — Dogbane Family

Apocynum cannabinum L.
Hemp-dogbane; infrequent at edges of woods and fields; 3282; 4.

Araceae — Arum Family

Arisaema triphyllum (L.) Schott
Jack-in-the-pulpit; common in woods; 2770; 4.

Araliaceae — Ginseng Family

Panax quinquefolium L.
American ginseng; scarce in woods; 3221; 9.

Aristolochiaceae — Birthwort Family

Asarum canadense L.
Wild ginger; common in woods; 2772; 7.

Asteraceae — Aster Family

Achillea millefolium L.
Common yarrow; edges of woods and fields; 3089; non-native.

+ *Ambrosia artemisiifolia* L.
Common ragweed; edges of fields; 3198; na.

Aster cordifolius L.
Common blue heart-leaved aster; frequent, margin of woods; 3213; 7.

Aster lateriflorus (L.) Britton
Goblet-aster; frequent, especially near margin of woods; 3233B; 4.

Aster novae-angliae L.
New England aster; scarce in old field; 3609; na.

Aster pilosus Willd.
Awl-aster; frequent at edge of fields; 3215; na.

Aster shortii Lindley
Midwestern blue heart-leaved aster; infrequent in woods; 3230; 8.

+ *Chrysanthemum leucanthemum* L.
Ox-eye daisy; edges of woods and fields; 3092; non-native.

+ *Cirsium arvense* (L.) Scop.
Canada thistle; edges of fields; 3088; non-native.

+ *Erigeron annuus* (L.) Pers.
Annual fleabane; old field and edge of woods; 3018; na.

Erigeron strigosus Muhl.
Rough fleabane; edges of fields; 3125; na.

Eupatorium purpureum L.
Purple-node joe-pye weed; scarce in woods; 3112; 7.

Eupatorium rugosum Hout.
White snakeroot; occasional in woods; 3201; 4.

Euthamia graminifolia (L.) Nutt. [*Solidago graminifolia*]
Common flat-topped goldenrod; edge of field; 3214; na.

Helianthus tuberosus L.
Jerusalem artichoke; frequent at edge of woods; 3197; 3.

Lactuca biennis (Moench) Fernald
Tall blue lettuce; infrequent at edges of woods; 3199; 4.

Lactuca floridana (L.) Gaertn.
Woodland lettuce; scarce in woods; 3232; 5.

Polymnia canadensis L.
Pale-flowered leaf-cup; infrequent in mesic woods; 3388; 10.

Prenanthes altissima L.
Tall white lettuce; scarce in woods; 3223; 8.

Rudbeckia hirta L.
Black-eyed Susan; local in old field; 3611; na.

Solidago caesia L.
Axillary goldenrod; frequent in mesic woods; 3212; 7.

Solidago canadensis L.
Common goldenrod; frequent at edge of field; 3187; na.

Solidago flexicaulis L.
Zigzag goldenrod; frequent in mesic woods; 3226; 7.

\+ *Taraxacum officinale* Weber
Common dandelion; fallow field; 2803; non-native.

Balsaminaceae — Touch-Me-Not Family

Impatiens capensis Meerb.
Orange touch-me-not; frequent near wooded depressions; 3130; 3.

Berberidaceae — Barberry Family

Caulophyllum thalictroides (L.) Michx.
Blue cohosh; infrequent in mesic woods; 3069; 8.

\+ *Podophyllum peltatum* L.
May-apple; infrequent in mesic woods; 2842; 4.

Betulaceae — Birch Family

Carpinus caroliniana Walter
Hornbeam; frequent in woods; 3302; 8.

Ostrya virginiana Walt.
Hop-hornbeam; frequent in woods; 3275; 4.

Bignoniaceae — Trumpet-Creeper Family

Campsis radicans (L.) Seemann
Trumpet creeper; scarce near wooded depressions; 3128; non-native.

Brassicaceae — Mustard Family

Barbarea vulgaris Br.
Yellow rocket; edge of cultivated field; 3147; non-native.

+ *Capsella bursa-pastoris* (L.) Medic.
Shepherd's purse; old field; 3259; non-native.

Cardamine concatenata (Michx.) Schwarz [*Dentaria laciniata*]
Five-parted toothwort; abundant in mesic woods; 2795; 5.

Cardamine douglasii Britton
Pink spring-cress; frequent in woods; 2775; 7.

+ *Cardamine rhomboidea* (Pers.) DC. [*C. bulbosa*]
Spring-cress; scarce in wet woods; 2850; 6.

Caesalpiniaceae — Caesalpinia Family

+ *Gymnocladus dioica* (L.) Koch
Kentucky coffee-tree; single individual sighted but also reported on Fox Island (Oliphant, 1988); 3136; 8.

Campanulaceae — Bellflower Family

Campanula americana L.
Tall bellflower; occasional in woods; 3123; 3.

Lobelia inflata L.
Indian tobacco; scarce in old field; 3610; na.

Lobelia siphilitica L.
Great lobelia; occasional, low edges of woods; 3200; 6.

Caprifoliaceae — Honeysuckle Family

+ *Lonicera morrowii* Gray
Honeysuckle; frequent, wood edges; 3139, 3239B; non-native.

Sambucus canadensis L.
Common elderberry; infrequent in wet woods; 3065; 1.

Viburnum acerifolium L.
Dockmackie; infrequent in woods; 3067; 9.

Viburnum prunifolium L.
Black haw; frequent, especially along edge of woods; 3218; 5.

Caryophyllaceae — Pink Family

+ *Cerastium nutans* Raf.
Mouse-ear chickweed; fallow field; 2800; na.

Cerastium vulgatum L. [*C. fontanum*]
Mouse-ear chickweed; fallow field; 2802; non-native.

Silene virginica L.
Fire-pink; infrequent, wood edge; 3033; 10.

+ *Stellaria media* (L.) Villars
Chickweed; fallow field; 2801; non-native.

Celastraceae — Staff-Tree Family

Euonymous obovatus Nutt.
Running strawberry-bush; scarce in damp woods; 3225; 7.

Chenopodiaceae — Goosefoot Family

Atriplex patula L.
Spearscale; infrequent at edge of cultivated field and woods; 3612; non-native.

Clusiaceae — Mangosteen Family

Hypericum majus (Gray) Britton
St. John's-wort; old field; 3099; na.

+ *Hypericum mutilum* L.
St. John's-wort; low edge of cultivated field; 3146; na.

Hypericum punctatum Lam.
Spotted St. John's-wort; old field; 3100; na.

Convolvulaceae — Morning-Glory Family

+ *Ipomoea purpurea* (L.) Roth
Common morning-glory; edge of cultivated field; 3190; non-native.

Cornaceae — Dogwood Family

+ *Cornus drummondii* Meyer
Rough-leaved dogwood; infrequent at wood edges; 3097; 2.

+ *Cornus florida* L.
Flowering dogwood; occasional in woods; 3211; 8.

Cuscutaceae — Dodder Family

Cuscuta gronovii Willd.
Common dodder; infrequent on *Laportea canadensis*; 3224; 4.

Cyperaceae — Sedge Family

+ *Carex albursina* Sheldon
Sedge; frequent, mesic woods; 2837; 7.

Carex blanda Dewey
Sedge; occasional, mesic woods and edges; 2977; 1.

+ *Carex bromoides* Willd.
Sedge; infrequent at edges of wooded depressions; 2972; 10.

Carex cephalophora Muhl.
Sedge; infrequent in margins of woods and old fields; 3263; 3.

+ *Carex conjuncta* Boott
Sedge; edge of woods and near the northern edge of its range; 2988; 10.

Carex davisii Schw. & Torr.
Sedge; woods, especially near margins; 3261; 7.

+ *Carex gracilescens* Steudel
Sedge; scarce, mesic woods; 3260; 10.

Carex gracillima Schw.
Sedge; infrequent near wooded depressions; 2976; 10.

Carex granularis Willd.
Sedge; infrequent at wood edges; 3284; 4.

Carex grayi Carey
Sedge; infrequent in wet woods; 3129; 7.

Carex grisea Wahl.
Sedge; occasional in woods; 2964; 2.

Carex hirtifolia Mack.
Sedge; frequent in mesic woods; 2832; 5.

Carex jamesii Schw.
Sedge; infrequent in mesic woods; 2827; 5.

Carex laxiculmis Schw.
Sedge; occasional in mesic woods; 2974; 10.

+ *Carex laxiflora* Lam.
Sedge; occasional in mesic woods; 2851; 10.

Carex lupulina Willd.
Sedge; infrequent near wooded depressions; 3080; 7.

+ *Carex molesta* Mack. *ex* Bright
Sedge; scarce at wood edge; 3285; 2.

Carex muskingumensis Schw.
Sedge; infrequent at edges of wooded depressions; 3062; 8.

Carex normalis Mack.
Sedge; infrequent at edges of woods; 3278; 5.

Carex pensylvanica Lam.
Sedge; infrequent in drier woods; 2844; 5.

Carex radiata (Wahl.) Small [*C. rosea*]
Sedge; infrequent near wooded depressions; 2971; 6.

+ *Carex rosea* Willd. [*C. convoluta*]
Sedge; occasional in woods; 2970; 4.

Carex shortiana Dewey
Sedge; edge of woods; 2987; 10.

Carex sparganioides Willd.
Sedge; frequent in mesic woods; 2965; 3.

Carex squarrosa L.
Sedge; infrequent near wood edges; 3277; 10.

Carex stipata Muhl.
Sedge; infrequent at wet wood edges; 3280; 3.

Carex vulpinoides Michx.
Sedge; infrequent at wood edges; 3084; 2.

+ *Carex woodii* Dewey
Sedge; locally frequent in woods; 2786; 10.

+ *Cyperus strigosus* L.
False nutsedge; edge of field; 3188; na.

Eleocharis ovata (Roth.) Roem & Schul.
Blunt spike-rush; low edge of cultivated field; 3148; na.

Dioscoreaceae — Yam Family

Dioscorea villosa L.
Colic-root; infrequent in mesic woods; 3228; 7.

Euphorbiaceae — Spurge Family

Acalypha rhomboidea Raf.
Rhombic copperleaf; occasional near margins of woods; 3203; 0.

Fabaceae — Pea Family

+ *Desmodium glutinosum* (Muhl. *ex* Willd.) Wood
Cluster-leaf tick-trefoil; occasional in woods; 3076; 5.

+ *Trifolium hybridum* L.
Alsike clover; edge of woods and fallow field; 3013; non-native.

+ *Trifolium pratense* L.
Red clover; edge of woods and fallow field; 3014; non-native.

+ *Trifolium repens* L.
White clover; edge of woods and fallow field; 3012; non-native.

Fagaceae — Beech Family

Fagus grandifolia Ehrh.
American beech; common in woods; 3231; 5.

Quercus alba L.
White oak; frequent in woods; 3020; 5.

Quercus bicolor Willd.
Swamp white oak; frequent in wet woods; 3296; 6.

+ *Quercus macrocarpus* Michx.
Bur-oak; occasional in woods; 3230B; 5.

Quercus muhlenbergii Engelm.
Yellow oak; infrequent near edges of woods; 3614; 8.

Quercus rubra L.
Northern red oak; common in mesic woods; 3137; 7.

Quercus velutina Lam.
Black oak; infrequent in western part of woods; 3288; 6.

Fumariaceae — Fumitory Family

Dicentra canadensis (Goldie) Walp.
Squirrel-corn; occasional in woods; 2797; 8.

Dicentra cucullaria (L.) Bernh.
Dutchman's breeches; occasional in woods; 2784; 6.

Geraniaceae — Geranium Family

Geranium maculatum L.
Wild geranium; abundant in woods; 2777; 4.

Grossulariaceae — Gooseberry Family

Ribes cynosbati L.
Dogberry; common in woods; 2788; 5.

Hippocastanaceae — Horse-Chestnut Family

Aesculus glabra L.
Ohio buckeye; frequent in woods; 2789; 3.

Hydrophyllaceae — Waterleaf Family

Hydrophyllum appendiculatum Michx.
Biennial waterleaf; frequent in woods; 2848; 8.

Hydrophyllum canadense L.
Maple-leaved waterleaf; occasional in woods; 3023; 10.

Hydrophyllum macrophyllum Nutt.
Hairy waterleaf; abundant in woods; 3022; 6.

Hydrophyllum virginianum L.
Eastern waterleaf; common in woods; 2979; 5.

Juglandaceae — Walnut Family

Carya cordiformis (Wang.) Koch.
Bitternut-hickory; infrequent in woods; 3208; 7.

Carya ovata (Miller) Koch.
Shagbark-hickory; frequent at edges of woods; 3217; 5.

Juglans nigra L.
Black walnut; frequent in woods; 3138; 5.

Juncaceae — Rush Family

Juncus tenuis Willd. var. *tenuis*
Path-rush; edges of woods and old field; 3086; 0.

Lamiaceae — Mint Family

Agastache neptoides (L.) Kuntze.
Catnip giant-hyssop; local in woods; 3111; 5.

Collinsonia canadensis L.
Northern horse-balm; frequent in mesic woods; 3116; 10.

Lycopus americanus Muhl.
American water-horehound; rare at moist field-edge; 3149; na.

Prunella vulgaris L.
Self-heal; edges of woods and fields; 3096; non-native.

Scutellaria lateriflora L.
Skullcap; scarce, damp wooded opening; 3617; 5.

+ *Stachys cordata* Riddell [*S. riddellii*, *S. nutallii*]
Hedge-nettle; infrequent in mesic woods; 3074; 10.

Teucrium canadense L. var. *virginicum* (L.) Eaton
American germander; occasional, wood edges; 3140; 3.

Lauraceae — Laurel Family

Lindera benzoin (L.) Blume
Spice-bush; abundant in moist woods; 2790, 3110; 7.

Liliaceae — Lily Family

Allium tricoccum Aiton var. *burdickii* Hanes
Ramps; rare in mesic woods; 2780, 3238B; 7.

Erythronium albidum Nutt.
White trout-lily; scarce in woods; 2794; 5.

Polygonatum pubescens (Willd.) Pursh
Solomon's seal; infrequent in woods; 2838; 7.

Smilacina racemosa (L.) Desf.
False Solomon's seal; abundant in woods; 2831; 3.

+ *Trillium flexipes* Raf. [*T. gleasoni*]
Bent trillium; infrequent in mesic woods; 2782; 6.

Trillium grandiflorum (Michx.) Salisb.
Big white trillium; abundant in mesic woods; 2776; 8.

Trillium sessile L.
Toadshade; infrequent in woods; 2773; 10.

Uvularia grandiflora Smith
Bellwort; frequent in mesic woods; 2771, 3024; 7.

Limnanthaceae — Meadow-Foam Family

+ *Floerka proserpinacoides* Willd.
False mermaid; infrequent in woods; 2774; 8.

Menispermaceae — Moonseed Family

Menispermum canadense L.
Moonseed; infrequent in moist, wooded openings; 3142; 6.

Moraceae — Mulberry Family

+ *Morus rubra* L.
Red mulberry; infrequent near wooded depressions; 3106; 10.

Oleaceae — Olive Family

Fraxinus americana L.
White ash; frequent in woods and edges; 3210; 5.

+ *Fraxinus nigra* Marsh
Black ash; infrequent in wet woods; 3135; 10.

Fraxinus pennsylvanica Marsh.
Green ash; scarce in wet woods; 3295; 5.

Onagraceae — Evening Primrose Family

Circaea lutetiana L. [*C. quadrisulcata*]
Common enchanter's nightshade; frequent in woods; 3082; 1.

Orobanchaceae — Broom-Rape Family

Epifagus virginiana (L.) Barton
Beech-drops; occasional in woods; 3613; 10.

Oxalidaceae — Wood-Sorrel Family

Oxalis dillenii Jacq.
Southern yellow wood-sorrel; fallow field and edge of woods; 3019; na.

Oxalis stricta L.
Common yellow wood-sorrel; edge of woods; 3081; 0.

Papaveraceae — Poppy Family

Sanguinaria canadensis L.
Bloodroot; occasional in woods; 2779; 6.

Phytolaccaceae — Pokeweed Family

Phytolacca americana L.
Pokeweed; scarce near wood edge; 3141; 1.

Plantaginaceae — Plantain Family

+ *Plantago lanceolata* L.
English plantain; edges of woods and fields; 3090; non-native.

Plantago rugelii Desne.
American plantain; edges of woods and fields; 3091; 0.

Poaceae — Grass Family

+ *Brachyelytrum erectum* (Schreber) Beauv.
Long-awned wood grass; occasional, moist, humus-rich soil in woods; 3375; 10.

+ *Bromus inermis* Leysser
Smooth brome; locally frequent at edges of woods and fields; 3286; non-native.

+ *Bromus pubescens* Muhl. [*B. purgans*]
Brome grass; infrequent in damp woods; 3387; 5.

+ *Bromus tectorum* L.
June grass; old field; 3257; non-native.

Cinna arundinacea L.
Common woodreed; frequent in woods; 3235; 5.

Dactylis glomerata L.
Orchard-grass; edge of woods and old fields; 2989; non-native.

+ *Digitaria ischaemum* (Schweig.) Muhl.
Smooth crab-grass; edge of woods and fields; 3196; non-native.

Echinochloa crusgalli (L.) Beauv.
Barnyard-grass; scarce in moist, wooded pathway; 3371; 0.

Elymus hystrix L. [*Hystrix patula*]
Bottlebrush-grass; common in mesic woods; 3057; 5.

Elymus villosus Willd.
Downy wild rye; scarce in damp woods; 3389; 5.

Festuca elatior L.
Tall fescue; edge of woods and old fields; 2990; non-native.

Festuca subverticillata (Pers.) Alexeev. [*F. obtusa*]
Nodding fescue; frequent in mesic woods; 3032; 5.

Glyceria striata (Lam.) Hitchc.
Fowl-mannagrass; scarce in damp woods; 3390; 4.

+ *Lolium perenne* L.
Ryegrass; old field; 3256; non-native.

Muhlenbergia schreberi Gmelin
Nimblewill; infrequent in damp woods; 3202; 0.

Poa pratensis L.
Kentucky bluegrass; edge of woods and old fields; 2986; non-native.

Poa sylvestris Gray
Forest bluegrass; occasional in damp woods; 2973; 10.

+ *Setaria faberi* Hermm.
Nodding foxtail; edge of cultivated field; 3195; non-native.

Polemoniaceae — Phlox Family

Phlox divaricata L.
Forest-phlox; abundant in woods; 2768; 5.

Polemonium reptans L.
Spreading Jacob's ladder; frequent in woods; 2778; 5.

Polygonaceae — Smartweed Family

+ *Polygonum persicaria* L.
Lady's thumb; edge of cultivated field; 3191; non-native.

Polygonum virginianum L.
Jumpseed; abundant in woods; 3115; 2.

Rumex crispus L.
Curly dock; fallow field; 3015; non-native.

Portulacaceae — Purslane Family

Claytonia virginica L.
Spring-beauty; occasional in woods; 2767; 2.

Primulaceae — Primrose Family

Lysimachia ciliata L.
Fringed loosestrife; infrequent in moist woods; 3063; 4.

Ranunculaceae — Buttercup Family

+ *Actaea alba* (L.) Miller [*A. pachypoda*]
Doll's eyes; infrequent in woods; 2830, 3071; 7.

Anemone quinquefolia L.
Wood-anemone; scarce in woods; 2833; 7.

+ *Anemonella thalictroides* (L.) Spach [*Thalictrum thalictroides*]
Rue-anemone; infrequent, mesic woods; 2839; 7.

Hepatica americana (DC) KerGawler
Round-lobed hepatica; infrequent, mesic woods; 2787; 9.

Hydrastis canadensis L.
Golden seal; scarce in mesic woods; 2791; 8.

Ranunculus abortivus L.
Small-flowered crowfoot; fallow field; 2799; na.

Ranunculus hispidus Michx. var. *caricetorum* (Greene) Duncan [*R. septentrionalis*]
Hispid buttercup; frequent near wooded depressions; 2783; 5.

Rosaceae — Rose Family

Agrimonia pubescens Wallr.
Downy agrimony; occasional, mesic woods; 3143; 5.

Crataegus mollis (T. & G.) Scheele
Downy hawthorn; wood edges; 3093; 2.

Crataegus punctata Jacq.
Dotted hawthorn; wood edges; 3276; 2.

Fragaria virginiana Duchesne
Thick-leaved wild strawberry; edge of woods and old field; 3017; 1.

Geum canadense Jacq.
White avens; frequent in mesic woods; 3120; 1.

Geum vernum (Raf.) T. & G.
Spring avens; occasional in woods; 2855; 5.

Potentilla norvegica L.
Strawberry-weed; old field; 3098; na.

Potentilla simplex Michx.
Old field five-fingers; infrequent, edge of woods and old fields; 3266; 4.

Prunus serotina Ehrh.
Wild black cherry; frequent at edge of woods; 2985, 3085; 1.

+ *Rosa multiflora* Thunb. *ex* Murr.
Multiflora-rose; infrequent, edge of woods; 3016; non-native.

Rosa setigera Michx.
Climbing prairie-rose; edge of woods; 3103; 7.

Rubus allegheniensis Porter
Common blackberry; edge of woods; 3031; 3.

+ *Rubus occidentalis* L.
Black raspberry; edge of woods; 2984; 2.

Rubiaceae — Madder Family

Galium aparine L.
Cleavers; abundant in woods; 2968; 1.

Galium circaezans Michx.
Forest bedstraw; infrequent in woods; 3227; 10.

Galium concinnum T. & G.
Shining bedstraw; occasional in woods; 3235B; 5.

Galium obtusum Bigelow
Bluntleaf-bedstraw; occasional in woods; 3234B; 5.

Rutaceae — Rue Family

Zanthoxylum americanum Miller
Common prickly-ash; edge of woods; 2829; 3.

Salicaceae — Willow Family

Populus deltoides Marsh
Cottonwood; infrequent at wet wood edge; 3294; 2.

Saxifragaceae — Saxifrage Family

Mitella diphylla L.
Two-leaved mitrewort; frequent, mesic woods; 2781; 10.

Penthorum sedoides L.
Ditch-stonecrop; scarce on a moist, wooded pathway; 3373; 5.

Scrophulariaceae — Figwort Family

Chelone glabra L.
White turtlehead; scarce in moist woods; 3222; 8.

+ *Collinsia verna* Nutt.
Blue-eyed Mary; local in flatwoods; 2793; 10.

Gratiola neglecta Torr.
Hedge-hyssop; local in moist, wooded pathway; 3290; 7.

Mimulus alatus Aiton
Sharpwing monkey-flower; damp, wooded opening; 3616; 7.

Penstemon digitalis Nutt.
Tall white beard-tongue; infrequent at edges of woods; 3281; 4.

Scrophularia marilandica L.
Eastern figwort; infrequent, damp woods; 3382; 4.

Veronica serpyllifolia L.
Thyme-leaved speedwell; fallow field; 2858; non-native.

Smilacaceae — Catbrier Family

Smilax ecirrhata (Engelm.) Wats.
Catbrier; frequent in mesic woods; 3268, 3301; 5.

Smilax herbacea L. var. *lasioneura* (Sm.) Rydb.
Catbrier; infrequent in mesic woods; 2852; 5.

Smilax hispida Muhl.
Bristly catbrier; infrequent in mesic woods; 3273; 5.

Solanaceae — Nightshade Family

Solanum nigrum L.
Black nightshade; infrequent in damp woods; 3380; 0.

Tiliaceae — Linden Family

Tilia americana L.
Basswood; common in woods; 3300; 5.

Ulmaceae — Elm Family

Celtis occidentalis L.
Northern hackberry; infrequent in moist, wooded openings; 3205; 3.

Ulmus americana L.
American elm; infrequent at wood edges; 3299; 3.

Ulmus rubra Muhl.
Slippery elm; common in moist, wooded openings; 3204; 4.

Urticaceae — Nettle Family

Boehmeria cylindrica (L.) Swartz
False nettle; infrequent in damp woods; 3234; 2.

Laportea canadensis (L.) Wedd.
Wood nettle; abundant in woods; 3121; 3.

Verbenaceae — Vervain Family

Phryma leptostachya L.
Lopseed; frequent in mesic woods; 3107; 4.

Violaceae — Violet Family

+ *Viola pubescens* Aiton
Yellow forest-violet; common in woods; 2769, 2975; 5.

Viola sororia Willd.
Dooryard-violet; infrequent in woods; 2798; 3.

Viola striata Aiton
Creamy violet; occasional in woods; 2962; 6.

Vitaceae — Grape Family

+ *Parthenocissus quinquefolia* (L.) Planchon
Virginia-creeper; common in woods; 3030; 2.

+ *Vitis vulpina* L.
Frost-grape; infrequent at edges of woods; 3194; 9.

Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 291-298

SILICA-SCALED CHRYSOPHYTES FROM INDIANA. II.

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ABSTRACT: A total of 32 scaled chrysophytes (Synurophyceae: 19 *Mallomonas* spp. and 6 *Synura* spp.; Chrysophyceae: 3 *Chrysosphaerella* spp., 2 *Spiniferomonas* spp., and 2 *Paraphysomonas* spp.) were identified from water samples from Lake Monroe, Monroe County, Indiana, using TEM. Twenty-two taxa were new records from Indiana, including *Paraphysomonas runcinifera*, a species new to North America.

KEYWORDS: Lake Monroe, *Paraphysomonas runcinifera*, silica-scaled chrysophytes.

INTRODUCTION

The first published electron micrographs of silica-scaled chrysophytes from Indiana were those of Wujek and Swinehart (1995). All previous reports were based on light microscopy. Kristiansen (1979) demonstrated that electron microscopy is needed to confirm chrysophyte identifications. The chrysophyte flora of Michigan is becoming well studied using the electron microscope (see Wujek and Igoe, 1989, and the literature therein). In this study, we used transmission electron microscopy to examine the silica-scaled algal flora from Lake Monroe, Monroe County, Indiana.

MATERIALS AND METHODS

Lake Monroe is located in south-central Indiana; the lake lies predominately in Monroe County but also extends into Brown, Jackson, and Lawrence Counties. Whole water samples from Lake Monroe (Table 1) were collected and concentrated by settling. These samples were fixed in M3 (Standard Methods, 1989). For transmission electron microscopy (TEM), subsamples were placed on Formvar-coated, carbon-stabilized grids, air dried, and examined with a Philips 300 TEM.

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Table 1. Physicochemical collection data for silica-scaled chrysophytes from Lake Monroe, Indiana, in 1994.

Sample Number	Site	ID	Depth (m)	pH	Temp (C)	Conductivity (μS/M)	Turbidity (NTU)[1]	Dis. Org. Carbon[2]	Dis. Nitrates and Nitrites[2]	Total Organic Carbon[2]
4 May										
1	MNR20003	87353*	Surface	7.0	15.3	173	14.4	5	0.2	3.91
2	MNR20003	87354	1.5	7.0	15.3	174	14.8	6	0.2	4.67
3	MNR21004	87358	Surface	6.9	15.4	189	14.5	5	0.3	4.55
4	MNR21004	87359	1.5	6.0	13.2	188	25.4	6	0.2	4.74
5	MNR21004	87361	6	6.8	26.9	172	49.3	6	0.3	5.06
25 May										
6	MNR20047	97103	3	6.7	19.1	123	7.2	6	0.0	5.55
7	MNR20047	97105	6	6.5	16.5	123	14.3	6	0.1	5.39
7 June										
8	MNR21001	95052	Surface	—	—	—	—	—	—	—
8 June										
9	MNR21004	95179	6	6.8	24.8	161	21.0	5	0.0	6.01
10	MNR20047	95183	Surface	7.5	26.8	150	19.8	5	0.0	5.13
11	MNR20047	95189	1.5	6.7	26.8	151	—	6	0.0	5.35
28 June										
12	MNR20003	96465	3	7.6	26.6	143	17.6	3	0.0	3.78
3 August										
13	MNR20003	96116	3	7.5	26.5	162	19.2	5	0.1	5.6
16 August										
14	MNR20004	96208	Surface	6.8	24.0	181	26.7	10	0.0	7.3

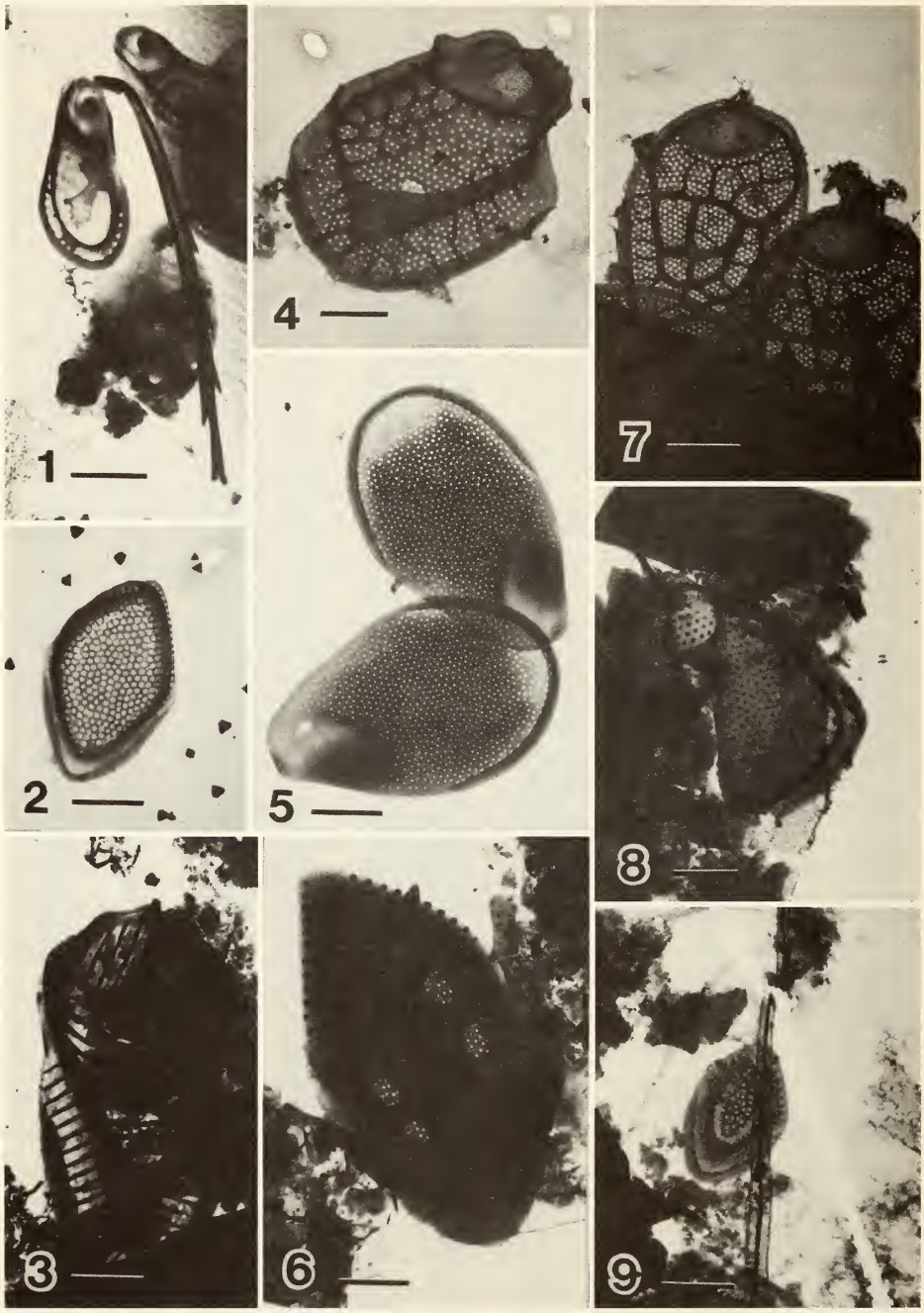
[1] National Turbidity Units.
[2] mg/L.
* Sample number of the Louisville District Army Corp of Engineers, Louisville, Kentucky.

OBSERVATIONS AND DISCUSSION

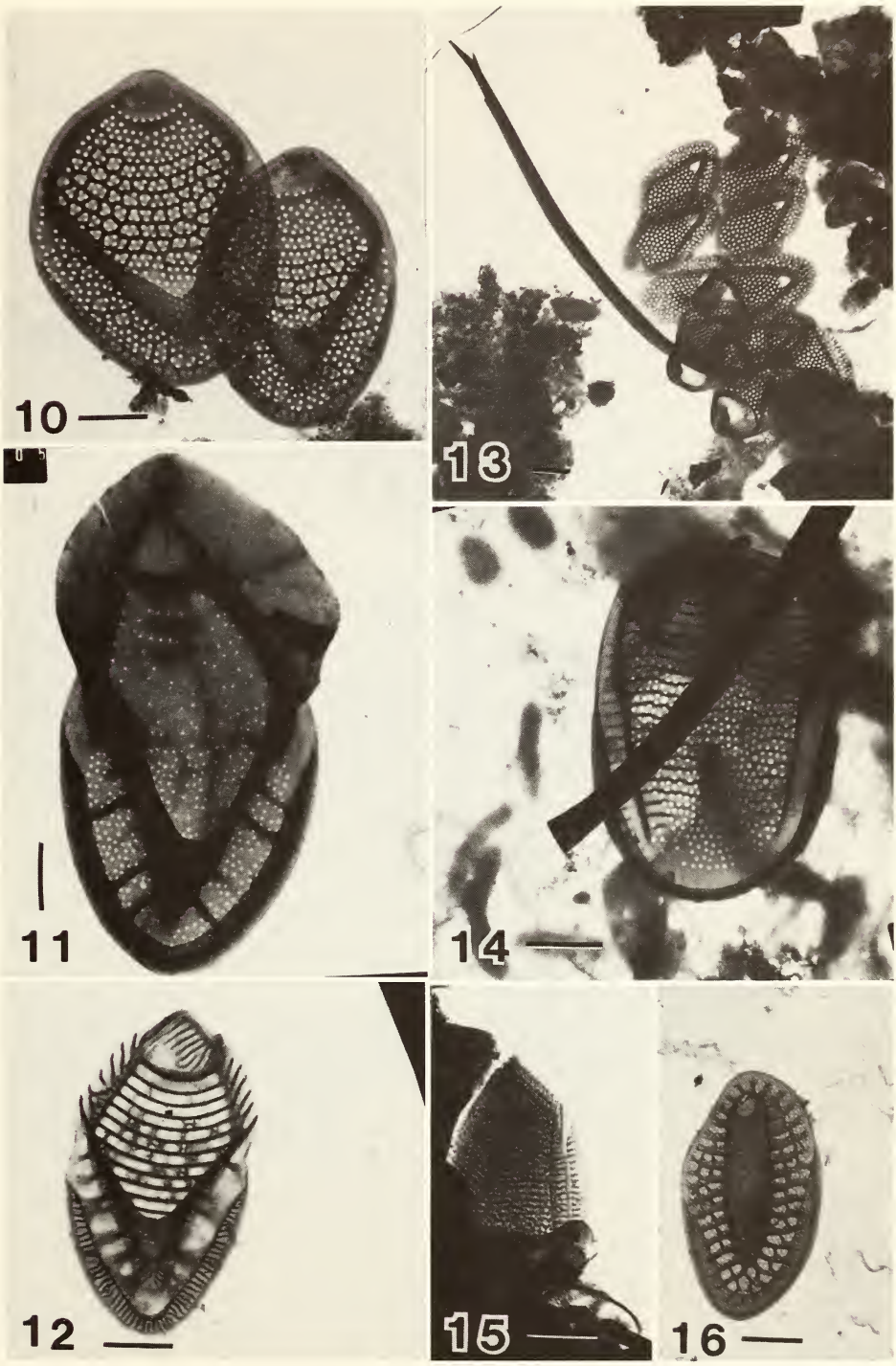
The taxa identified from Monroe Lake are listed in Table 2. Physiochemical parameters are listed for each sample in Table 1. Taxa reported for the first time from Indiana are marked with an asterisk. A total of 32 silica-scaled chrysophyte taxa from the genera *Mallomonas* (19 taxa), *Synura* (6 taxa), *Chrysosphaerella* (3 taxa), *Spiniferomonas* (2 taxa), and *Paraphysomonas* (2 taxa) were observed (Figures 1-23; only species not previously reported from Indiana based on electron microscopy are illustrated). Because the collections were all made during the summer, we suspect that reports of additional species

Table 2. Survey of the occurrence of silica-scaled chrysophytes in Lake Monroe, Indiana. See Table 1 for descriptions of sample sites and dates. Open circles indicate that both isolated scales and complete cells were observed. An asterisk is a new report for Indiana based on electron microscopy.

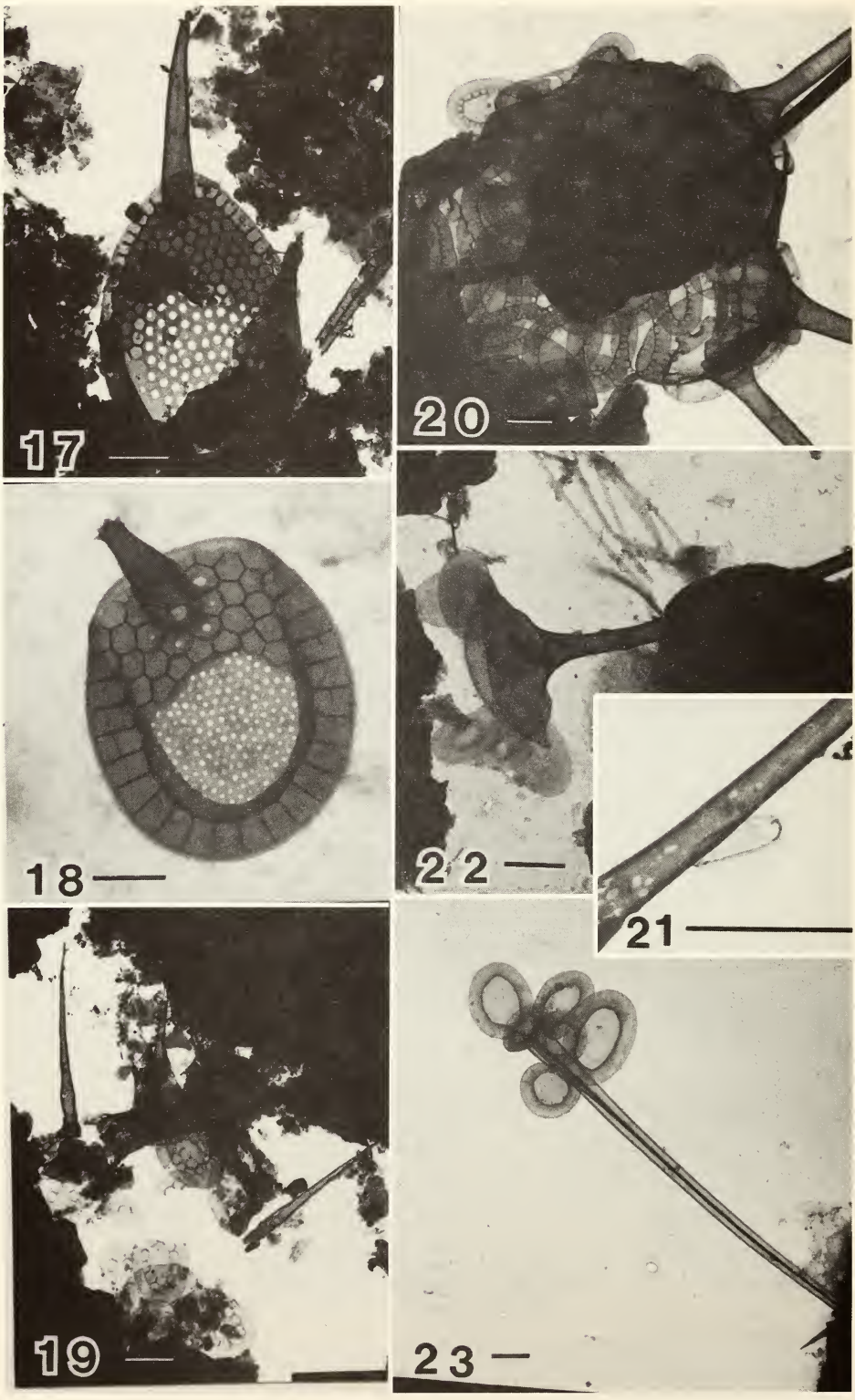
Taxon	Sample Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of Taxa/Collection	10	19	21	13	8	17	7	4	15	6	8	10	6	6
Synurophyceae														
Mallomonas														
M. acaroides	●	●	●											
**M. akrokomos*			○			○			○			●		●
**M. annulata*		●	●											
**M. asmundiae*			●											
M. caudata		●	●	●	●	●	●		●	●		●		
**M. crassisquama*	○	○	○	●	●	●	●	●	●	●	●	●	●	
**M. hamata*		○				●								
**M. heterospina*				●		●			●	●	●			
**M. lychenensis*		●												
M. mangofera f. *mangofera*				●		●			●					
**M. mangofera* f. *foveata*						●					●	●		
M. papillosa var. *papillosa*		●	●											
**M. pillula* var. *valdiviana*			●											
**M. portae-ferreae*		●	○									●		
**M. pseudocoronata*									●	●				
**M. striata* var. *serrata*			●											
**M. tonsurata*	○	●	○	●	●	●	●	●		○	●		●	●
**M. transsylvanica*	○	●	●	●	●	●			●					
**M.* sp. 1												●		
Synura														
S. curtispina	●	●	●	●	●	●			●				●	●
S. echinulata	○	●	●	●	○	○	○		●	●	●	●		
S. petersenii f. *petersenii*	○	●	○	●	●	○	●		●	●	●			
**S. petersenii* f. *kufferathi*				●										
**S. spinosa*		●							●				●	●
**S. uvella*			○				○							
Chrysophyceae														
Chrysosphaerella														
**C. annulata*									●			●		
C. brevispina	●	●	●			●			●					
**C. coronacircumspina*		○	●			●			●		●	●		
Paraphysomonas														
**P. runcinifera*		●												
P. vestita	●	●	●	●		●	●	●	●			●	●	●
Spiniferomonas														
**S. bourrellyi*			●	●		●								
**S. trioralis*	○	○	○	●	●	●		○			○		●	●



Figures 1-9. **1.** *Mallomonas akrokomos*. **2.** *M. annulata*. **3.** *M. asmundiae*. **4.** *M. crassisquama*. **5.** *M. hamata*. **6.** *M. lychenensis*. **7.** *M. heterospina*. **8.** *M. mangofera* var. *foveata*. **9.** *M. pillula* var. *valdiviana*. Scale bars = 1 μm.



Figures 10-16. **10.** *Mallomonas portae-ferreae*. **11.** *M. pseudocoronata*. **12.** *M. striata* var. *serrata*. **13.** *M. tonsurata*. **14.** *M. transsylvanica*. **15.** *M.* sp. 1. **16.** *Synura petersenii* f. *kufferathi*. Scale bars = 1 μm.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Figures 17-23 (opposite page). **17.** *Synura spinosa*. **18.** *S. uvella*. **19.** *Chrysosphaerella annulata*. **20.** *C. coronacircumspina*. **21.** *Paraphysomonas runcinifera*. **22.** *S. bourrellyi*. **23.** *S. trioralis*. Scale bars = 1 μm.

in this region will be forthcoming. One species, *Paraphysomonas runcinifera* (Figure 21), is new to North America.

The number of scale-bearing chrysophyte taxa observed per sample ranged from 6 to 21. Species richness was greatest at lower water temperatures (Table 1). The most frequent *Mallomonas* species in the study were *M. crassisquama* (86%) and *M. tonsurata* (86%), *M. caudata* (64%), and *M. transsylvanica* (50%). Common species from other genera were *Synura echinulata* (79%), S. petersenii f. *petersenii* (71%), *S. curtispina* (64%), *Paraphysomonas vestita* (79%), and *Spiniferomonas trioralis* (71%). Species observed only once included *Mallomonas annulata*, *M. asmundiae*, *M. lychenensis*, *M. pillula* var. *valdiviana*, and *Paraphysomonas runcinifera*. Not surprisingly, the highest diversities were observed in those samples having the greatest dissolved nitrates + nitrites (Table 1).

The range in temperature (13.2° to 26.9° C) and the time of year may have resulted in elements of both the late spring and summer floras being collected. Species that were common in this study, such as *Mallomonas akrokomos* and *M. transsylvanica*, have often been found under the ice in more northern regions (Siver, 1991; Cronberg and Kristiansen, 1980). On the other hand, taxa such as *M. crassisquama*, *M. tonsurata*, and *Synura curtispina*, are more commonly found during the summer (Siver, 1991), supporting the idea that our collections contain elements of both the summer and winter floras.

The pH ranged from 6.5 to 7.6. Many of the species observed during this study, including *Mallomonas hamata*, *M. mangofera* var. *foveata*, *M. transsylvanica*, *Synura echinulata*, and *Spiniferomonas alata*, have been previously reported as common in acidic habitats (Siver, 1988, 1989, 1991). Our observations support these earlier findings.

In conclusion, as has been demonstrated in other regions of the upper Midwest, Indiana contains a diverse flora of scaled chrysophytes. Lake Monroe's chrysophyte diversity was greater than that of the ten different Indiana water bodies previously reported by Wujek and Swinehart (1995). We believe that further collections and observations from other geographic areas in Indiana will yield additional species. Including the species reported in this paper, the scaled chrysophyte flora of Indiana now has 37 known taxa.

ACKNOWLEDGMENTS

The authors wish thank Dr. F.K. Daily (Butler University) for literature assistance, P.R. Elsner for preparing the carbon-coated grids, CMU Graphics Production for assistance in the preparation of the photographic plates, and Dr. Jørgen Kristiansen for commenting on several of our identifications. The Louisville Dis-

trict of the Army Corp of Engineers provided the phytoplankton samples and physiochemical data.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 299-306

I AM! THEREFORE, I VOTE! SELF-MONITORING AND 1996 PRESIDENTIAL VOTING CHOICES

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ABSTRACT: Two studies were conducted to examine the impact of the self-monitoring tendency on information processing and social choices. In study 1, high and low self-monitors were provided with "nonsense" words and asked to define each of them. High self-monitors defined more of the nonsense words as physical and social role descriptors, whereas low self-monitors defined significantly more of the words as trait descriptors. In Study 2, voters leaving polling centers during the 1996 Presidential Election were given the self-monitoring scale and asked to identify the Presidential candidate for whom they voted. High self-monitors overwhelmingly voted for Bill Clinton, while low self-monitors overwhelmingly endorsed Bob Dole. The rationale for these differences and suggestions for future research are discussed.

KEYWORDS: Information processing, person perception, self-monitoring, voting choices.

INTRODUCTION

The self-monitoring literature provides ample evidence of the fact that an individual's self-monitoring tendency influences how that individual will process social information and the choices he or she will make. Clearly, self-monitoring plays a role in determining how the individual will process and utilize social information as it pertains to self and others.

Self-monitoring level has been shown to predict the effects that self-monitoring propensities have on the processing of self-relevant information (Snyder and Cantor, 1980), choosing friends as activity partners (Snyder, Gangestad, and Simpson, 1983), orientations toward personnel selection (Snyder, Berscheid, and Matwychuk, 1988), and initiation of personal relationships (Snyder, Berscheid, and Glick, 1985). High self-monitors seem to pay particular attention to situational cues that suggest what behaviors are appropriate; they are aware of the social roles that are salient in a situation and are concerned with appearances (*e.g.,* Berscheid, Graziano, Monson, and Dermer, 1976; Snyder, Berscheid, and Glick, 1985; Snyder, Gangestad, and Simpson, 1983). Low self-monitors, on the other hand, monitor internal aspects of self and seem concerned with modulating behavior to fit with some internal standard. Low self-monitors also seem particularly interested in the internal characteristics of others, such as attitudes, values, and beliefs (*e.g.,* Snyder, Berscheid, and Glick, 1985; Snyder and Cantor, 1980; Snyder, 1987).

In social situations where choices are not obvious, implicit personality theories are commonly used to process information and make behavior choices (Bruner and Tagiuri, 1954; Cantor and Kihlstrom, 1986; Cantor and Mischel, 1979; Schneider, 1973). High self-monitors seem to have readily accessible stores of information about situations and appropriate behaviors (*e.g.,* Snyder and Cantor, 1980), whereas low self-monitors usually process information in trait terms. This difference in their mode of processing information coupled with the fact that individuals tend to process information in a self-relevant fashion (*e.g.,* Fong and Markus, 1982; Kuiper and Rogers, 1979; Lemon and Warren, 1974; Lewicki, 1983, 1984) strongly suggests that individuals may use their self-monitoring tendency for the processing of social information and for making social choices.

Ickes, Layden, and Barnes (1978) discovered that high self-monitors were particularly likely to use role relationship descriptors when asked to answer the question, "Who am I?" Sampson (1978) provided further evidence for the notion that high self-monitors differ in the categories they consider to be important in defining self. High self-monitors were found to rate externally located descriptors as being more important for their sense of self than internally located descriptors. Snyder (1995) goes on to show us that high self-monitors typically strive for behaviors that are appropriate to the social setting.

High self-monitors should pay particular attention to the outward, behavior-oriented aspects of the individual because they assume that the situation is most predictive of one's behavior. Low self-monitors, on the other hand, seem to believe that trait or character information is more informative as to what kind of person they are observing. As such, low self-monitors should pay particular attention to information about the internal traits of the person they are observing (Snyder and Cantor, 1980; Snyder, Gangestad, and Simpson, 1983; Snyder and Monson, 1975). Snyder (1995) suggests that low self-monitors characteristically seek, either through words or deeds, to faithfully express their attitudes, feelings, and personalities (*i.e.,* their inner selves). Based on this literature, Osborne, *et al.* (1996) argued that the self-monitoring tendency may become a heuristic individuals use to make sense of information in their social worlds.

This brief review of the findings in the self-monitoring literature indicates that high and low self-monitors have very different ideas as to what is informative about other people and the world around them. Thus, the construct of self-monitoring may be useful in determining the categories of information individuals will use to evaluate others, even if those others are not well known (Snyder, Berscheid, and Matwychuk, 1988; Young, Osborne, and Snyder, 1994).

STUDY 1

Introduction. Osborne, *et al.* (1996) showed that high and low self-monitors, when presented with social stimuli, will process those stimuli according to their self-monitoring tendency. High self-monitors recalled significantly more physical and social role descriptors from a target audiotape, while low self-mon-

itors recalled significantly more personality trait descriptors. If, as Osborne, *et al.* (1996) suggest, self-monitoring is automatically used by individuals to process information, self-monitoring should occur even with information that is novel. In previous studies, the information presented to the subjects was all social in nature. The very descriptors that were presented may have activated the self-monitoring tendency. However, if the self-monitoring tendency is utilized as a basic information processing strategy, this tendency should be utilized by participants even when the information being presented has no obvious meaning.

Study 1 represents an attempt to replicate the findings of Osborne, *et al.* (1996) and to provide stronger support for their contention that the self-monitoring tendency is utilized by individuals to make sense of incoming information. Participants were given an ostensible target description of twenty coded (nonsense) words. We predicted that high self-monitors would define more of the nonsense words as relating to physical and social role descriptions, whereas low self-monitors would define more of the nonsense words as relating to the target's personality traits.

Methods. Thirty-eight students at a small Midwestern university participated in this study to earn assignment points in their General Psychology course. Thirty-four of the participants were female, and four were male. Previous studies by Osborne and his colleagues suggest that no consistent gender findings characterize this line of research; therefore, gender analyses were not conducted (*e.g.,* Osborne, *et al.,* 1996; Young, *et al.,* 1994).

Participants were presented with a written version of the target description used by Osborne, *et al.* (1996). The written description was reformatted using a symbol coding program on a word processor. The result was twenty-one coded and underlined words within the text. The participants were told that the text was a description of another person and that the experimenters were interested in how different individuals interpret information with which they are not familiar. Participants were provided with a data sheet that listed the "nonsense" words in numerical order along with the following simple instructions:

> Read each of the following coded words carefully. After you have looked at the word, write down a definition for what you think that word might mean. Please remember that there are no right or wrong answers. Do not leave any item blank.

After completing the "decoding" task, the participants were administered an 18-item self-monitoring scale (Snyder and Gangestad, 1986). After completion of this scale, the participants were probed for suspicion, debriefed, thanked for their participation, and dismissed. None of the participants reported understanding the coded words beyond chance levels of certainty.

Results. The participants' self-monitoring scales were scored and placed into a frequency distribution. From this distribution, the mode was calculated. The modal score on the 18-item measure was eleven with five participants scoring at that level. In order to determine those scoring high or low on the self-mon-

Table 1. The mean number of "nonsense" words defined by category based on a subject's self-monitoring level.

	Descriptor Type		
Group	Physical	Social Roles	Traits
Low self-monitors	2.556[1]	2.944[2]	12.056[3]
High self-monitors	9.733[1]	5.267[2]	2.200[3]

[1] $t_{(1,31)} = -7.648, p < 0.01$.
[2] $t_{(1,31)} = -2.805, p < 0.01$.
[3] $t_{(1,31)} = 10.146, p < 0.01$.

itoring scale, the participants scoring at the modal level were removed from future analyses. Individuals scoring above the mode were labeled as high self-monitors, whereas those scoring below the mode were labeled as low self-monitors. This method of constructing low and high self-monitor groupings is consistent with the self-monitoring literature (*e.g.,* Snyder, 1987; Snyder, Gangestad, and Simpson, 1983). These categorical scores were used to determine groupings for the *t*-test.

The participants' written definitions of the coded words were scored by naive judges into four categories: physical descriptors, social role descriptors, personality trait descriptors, and other. The two judges agreed on 94% of the categorizations, and a third naive judge was used to decide any disagreements. These categorizations were analyzed using the *t*-test.

In previous studies, high self-monitors tended to utilize more physical and social role descriptors to describe self and others and also remembered more of these descriptors when asked to recall what they could remember about someone they had just met. Low self-monitors, on the other hand, consistently utilized trait-oriented descriptors to describe self and others and remembered more trait descriptors about someone they had just met (see Osborne, *et al.,* 1996). We predicted that the same pattern of results would emerge when high and low self-monitors defined "nonsense" words they believed were social in nature. *T*-test analyses of the raters' categorization of the participants' responses support this prediction (Table 1). High self-monitors categorized more of the nonsense words to be physical and social role descriptors than their self-monitoring counterparts. Low self-monitors defined more of the nonsense words as describing personality traits than their high self-monitoring counterparts.

Discussion. The findings from Study 1 support the contention of Osborne, *et al.* (1996) that high and low self-monitoring tendencies are utilized in basic information processing. Previous studies by Osborne and his colleagues either presented social information to high and low self-monitors and tested the patterns of recall or asked participants to generate descriptions of self or well-known others. These researchers argued that the consistent differences in recall or descriptors generated supported their assumption that the self-monitoring tendency is an automatically utilized information processing strategy.

In order to qualify as an "automatic information processing strategy," the self-monitoring tendency should be utilized by participants to process information that is not well known. Although the participants in the current study were informed that the nonsense descriptors were taken from a target description, no other clues were provided, and a priming effect seems significantly less likely than in the previous studies. Despite the participants' admissions that they

were just "guessing" as to what the words meant, the same differential pattern was found as was revealed in previous studies. Low and high self-monitors differed significantly on what they defined these nonsense words to be. This finding is consistent with the prediction of Osborne, *et al.* (1996) that high and low self-monitors would utilize their self-monitoring tendencies to make sense of their social world. The fact that the predicted pattern was replicated using "nonsense" information further supports the notion that high and low self-monitors will automatically utilize self-monitoring as an information processing strategy.

Given what Lewicki and others (*e.g.,* Carpenter, 1988; Fong and Markus, 1982; Lewicki, 1983, 1984) have discovered about the self-referent effect, these predictions make perfect sense. Since self is often salient in social interactions, the salient aspects of self are most likely be utilized as a reference point for processing information in a social setting. The current study replicates the earlier findings of Osborne and his colleagues (*e.g.,* Osborne, Penticuff, and Weadick, 1996) but extends those findings to information that is novel or unknown.

STUDY 2

Given the importance of social information processing, an understanding of the role of self-monitoring and other personality characteristics in the process is imperative. High and low self-monitors differ in the types of partners they choose for activities (Snyder, Gangestad, and Simpson, 1983), the political choices they make (Young, Osborne, and Snyder, 1994), and the types of advertisements they find convincing (*e.g.,* Snyder, 1979; Snyder and Cantor, 1980).

Young, Osborne, and Snyder (1994) provided high and low self-monitors with candidate portfolios. In pretest data collection, the experimenters gathered information from participants about what issues they felt were the most important in their political choices and what their stances were on those issues. When these participants were later provided with candidate portfolios, the self-monitoring tendency clearly influenced voting choice. High self-monitors consistently voted for the candidate that was rated by pilot test participants as "looking the most presidential" regardless of where that candidate stood on the issues. Low self-monitors, on the other hand, consistently voted for the candidate that matched them most closely on the issues regardless of how "presidential looking" that candidate had been rated by pilot test participants. Although this result is compelling evidence for the use of the self-monitoring tendency, few attempts have been made to apply such findings in situations where choices have clear, real-world implications.

In an attempt to replicate the findings of Young, Osborne, and Snyder (1994) and to generalize those findings to actual voting choices, voters leaving the polls during the 1996 Presidential Election were given the self-monitoring scale and asked to indicate the candidate for whom they had voted. We predicted that high self-monitors would be significantly more likely to vote for Bill Clinton because he was the current President and was clearly ahead in the polls. Who could fit the image of "President" more in the eyes of a high self-monitor than the incum-

Table 2. The influence of the self-monitoring tendency on voters' presidential choices. The numbers outside the parentheses are the observed frequencies. The numbers in parentheses are the expected frequencies based on the null hypothesis (chi-square = 52.84, $df = 1$, and $p < 0.01$)

	Presidential Choices	
Group	Clinton	Dole
High self-monitors	47 (31.5)	13 (28.5)
Low self-monitors	7 (22.5)	36 (20.5)

bent President and the candidate being touted by the media as the obvious winner? Our prediction was consistent with the findings that high self-monitors assess the situation and tend to adjust their behaviors to what is expected (*e.g.,* Berscheid, Graziano, and Dermer, 1976; Snyder, Berscheid, and Glick, 1985; Snyder, Gangestad, and Simpson, 1983).

We also predicted that low self-monitors would be significantly more likely to vote for Bob Dole. The media paid a lot of attention to Dole's years in office and his consistent stance on issues. Given the value that low self-monitors place on consistency, they should be more likely to vote for Dole because of his length of service. This prediction is consistent with previous findings in which low self-monitors make their choices based more on internally based information and not on what is situationally appropriate or more popular (*e.g.,* Snyder and Cantor, 1980; Snyder, Gangestad, and Simpson, 1983; Snyder and Monson, 1975).

Methods. The participants were 135 voters leaving the polls in a moderate-sized Midwestern city. Participation was strictly voluntary. Of the 135 voters approached, 114 agreed to participate. Of these 114 participants, 65 were male, and 49 were female. Their ages and other demographic information were not collected. Participants were asked to volunteer for a study on "voter characteristics and voter choices." If a participant agreed to participate, he or she was given the 18-item self-monitoring scale and then asked to identify the presidential candidate for whom he/she had voted.

Results. Of the 114 participants, eleven voted for Ross Perot. Given a relatively equal split between those participants on self-monitoring (six were high self-monitors, and five were low) and the small number of Perot voters, these participants were not used in the final analyses. The 103 participants who voted for either Clinton or Dole were categorized as either "high" (scores of nine or higher) or "low" (scores of eight or lower) self-monitors based on their scores on the 18-item self-monitoring scale.

A chi square analysis was conducted on the frequencies of the high and low self-monitors voting for each candidate. If the self-monitoring score is not related to voting choice, then we would expect a relatively equal number of both high and low self-monitors to vote for each candidate. A clear indication exists that high and low self-monitors differ in their voting choices (Table 2). The observed frequencies were analyzed using a chi-square test. The calculated value of chi-square was 52.84, a value that was significantly greater than the critical value of chi-square (6.64). The null hypothesis, which predicted that high and low self-monitors would not differ in their choice of a presidential candidate, can be rejected at the 0.01 level.

Discussion. These findings lend significant support to the argument that the self-monitoring tendency may be utilized by individuals as a basic information processing strategy. The fact that these findings have been replicated in several studies and illustrated in actual voting behavior is quite compelling. Given what we know about the preferences of high and low self-monitoring individuals, the reasons that these voters may make differential political choices makes sense. To the extent that one candidate fits the "image" of being President (either by virtue of physical looks or actually being the incumbent President), one can safely assume that high self-monitors will be more inclined to vote for that candidate. These individuals have been shown to utilize image in choosing the products they buy (Snyder, 1995), the individuals they choose as activity partners (Snyder, Gangestad, and Simpson, 1983), and the individuals whom they date (Snyder, Berscheid, and Glick, 1985). Their choice of a political candidate seems subject to the same tendencies.

Perhaps these differential effects are more a function of the individual's apparent tendency to utilize the self-monitoring tendency as a basic information processing strategy than any specific, conscious, decision-making process. Very few individuals make a conscious decision to vote for a presidential candidate because he "looks like a President," yet the differential voting choices of high and low self-monitors were striking.

Low self-monitors have been shown to be more internally focused in making choices. They choose activity partners based on their feelings toward that person and not on how the person looks or on how good he/she might be at some required skill (Snyder, Gangestad, and Simpson, 1983); they date individuals who are similar to themselves in terms of their attitudes and beliefs (Snyder, Berscheid, and Glick, 1985); and they show preferences for products that stress quality and not image (Snyder, 1995). These same tendencies also influence the political choices that low self-monitors make.

When more detailed questions were asked of high and low self-monitors (Young, Osborne, and Snyder, 1994), even more interesting patterns emerged. High and low self-monitors were asked the following question, "If you were given $100 to donate to these candidates, how would you donate the money and why?" High self-monitors donated 100% of the money to their preferred candidate (shown in these studies to be the candidate pilot test participants rated as "looking the most presidential"). Low self-monitors divided their money equally between the candidates. When asked why they donated in this fashion, their answers were compelling. Many of the low self-monitoring participants indicated that they "donated money to the person I voted for because I like his position on the issues." In addition, many of these people went on to state that they "donated money to the other candidate because he will probably win."

Too little information was available to determine if equal giving was an attempt by low self-monitors to "hedge their bets" or whether they understood that the candidate that "looks presidential" is probably more likely to win. The degree to which these individuals were cognizant of why they made the choic-

es they did is certainly worth investigating. Future research should be directed toward this very phenomenon. If low and high self-monitors do make differential dating partner, activity partner, political, and product choices because of an automatic tendency to "filter" information through their self-monitoring tendency, the implications for how to approach, communicate, and present information to these individuals are profound.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 307-317

DO INDIANAPOLIS AIRPORT TEMPERATURES REPRESENT INDIANA'S ENERGY NEEDS?

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ABSTRACT: Air temperature data from the National Weather Service at the Indianapolis International Airport are used by the heating industry to estimate heating degree days and the corresponding heating fuel needs for most of Indiana. Although the airport is located southwest of the main metropolitan area (climatologically the windward side and least subject to urban heating), the city heat island may cause unrepresentatively high temperatures at the airport and correspondingly lower estimates of the heating fuel needs of Indiana. This concern is addressed in this paper. Temperature records from 1955 to 1993 at the airport were compared with those from the rural climatological station at Whitestown, about 19 miles north of the airport. The temperature variables examined for both stations were the January, July, and annual mean daily maximum and minimum temperatures and their respective differences. The airport-Whitestown differences for the July and mean annual daily maximum temperatures showed statistically significant upward time trends. Using the 1961-1990 temperature normals adjusted to a midnight observation base, the north-south gradients of the mean daily maximum and minimum temperatures for each month were compared with an average of those for three pairs of rural climatological stations. The mean daily temperatures at the airport averaged about 0.8° F higher for the year than those in rural settings. The corresponding annual heating degree day total was estimated to be about 251 heating degree days (or 4%) too low for predicting the heating needs of rural Indiana.

KEYWORDS: Heating degree days, heating fuel needs, Indianapolis airport, temperature gradients, time of observation temperature bias, urban heat islands.

INTRODUCTION

Air temperature data from the National Weather Service at the Indianapolis International Airport are used by the heating industry to predict the heating fuel needs for most of Indiana. The airport has 24-hour temperature records as well as the maximum and minimum for each day ending at midnight. Although the airport is located southwest of the city (climatologically the windward side and least subject to urban influences), some concern exists that the urban heat island and possibly the increasing airport runway pavement mass may cause unrepresentatively high temperatures at the airport and, therefore, lower estimates of the heating degree days and fuel needs than would be indicated by temperature data from rural climatological stations. Heating degree days (HDD) are derived from the daily mean temperatures $(\overline{T})$, assuming no heating needs when the daily mean temperature is 65° F or higher (*i.e.,* HDD = 65 - $\overline{T}$ and HDD = 0

for $\bar{T} \geqslant 65°$ F). The daily heating degree days are summed and correlated with fuel use for any period.

The existence of the urban heat island has been well documented (Landsberg, 1981; Oke, 1978). Although the effect of single residences, farm buildings, and small towns on climate is minor, larger towns and cities develop a climate of their own, due to the changes in atmospheric composition and energy balance caused by air pollution, concrete building and pavement masses, and decreased evaporation. Annual mean temperatures in cities average at least 1° to 2° C higher than those in surrounding rural areas; the greatest temperature differences occur in the winter heating season and in periods of calm, clear weather under stagnant high pressure cells.

Our overall objective in this study was to estimate the effect of the Indianapolis urban heat island on airport temperatures and the adjustments required to obtain "rural" daily mean temperatures and respective heating degree days for the airport. The representativeness of airport temperatures for predicting Indiana fuel needs can be estimated using at least two different approaches: 1) by comparing the observed fuel use with the usage predicted from the airport temperature and the derived heating degree days; and 2) by comparing the airport temperatures with those from adjacent rural climatological stations. Since we do not have the observed and predicted fuel use data, we are limited here to the second approach.

Since few, if any, climatological stations have homogeneous records over periods sufficiently long to provide unadjusted temperature "normals," our first objective was to examine the station histories, select the best rural control station, and adjust its records *a priori* for changes in location, instrumentation, and time of observation. Our second objective involved the comparison of airport temperature trends with those of the adjusted control station, using the daily maximum and minimum temperatures for January, July, and the year. Our last objective was to compare the north-south temperature gradients between the airport and the control station for each of the calender months with those of several other rural pairs in west-central and central Indiana.

DATA AND PROCEDURES

The Indianapolis International Airport is located on the southwestern corner of Indianapolis, and, with the exception of increasing runway pavements, nothing but grass and agricultural land occurs from south through west to north of the airport weather station. Given the prevailing southwesterly winds, little urban influence should occur, except for occasions of calm weather and air flow from the northeast to southeast, when urban warming might be expected. The air temperature at the airport was measured with a hygrothermometer (HO-60 to 1983 and HO-83 to the present) at the same exposure in the runway complex (1.8 m above sod) since 1 September 1959 (National Climatic Data Center, 1993, Indianapolis Station Location; personal communication, Roger Kenyon, Cooperative Program Manager, National Weather Service, and Jerry Reed, National

Weather Service Electronics Supervisor, Indianapolis, as well as Ann Lazar, National Climatic Data Center, Asheville, North Carolina). From 24 December 1954 to 1 September 1959, telepsychrometer equipment was used, effectively providing a homogenous record of maximum and minimum temperatures for days ending at midnight from 1955 to the present (a total of 39 years through 1993).

The rural climatological station selected for our control was Whitestown, Indiana. Whitestown lies 30 km north of the airport. While this rural station is in town (population 460), the station is surrounded by agricultural land and is considered a rural site. Epperson and Dale (1984) described this station as one of the most homogeneous climatological stations in Indiana, but a few minor moves did occur along with two changes in the time when the once-daily temperature observation was made. Epperson and Dale (1984) adjusted the 1909 to 1965 daily mean maximum and minimum temperatures from a 6 P.M. to a 7 A.M. observation day base (the 7 A.M. schedule began on 1 October 1965). On 1 September 1986, the liquid-in-glass maximum and minimum thermometers, which had been exposed in a Cotton Region Shelter since 1909, were replaced with an electronic Maximum-Minimum Temperature System and the observation time was changed from 7 A.M. to 5 P.M. EST. The change required adjusting the 1 September 1986 to December 1993 mean daily maximum and minimum temperatures at Whitestown to provide a homogeneous 7 A.M. observation day temperature through 1993. The change was made by the method described by Epperson and Dale (1984) using data from the West Lafayette 6NW Indiana climatological station at the Purdue University Agricultural Research Center located about 80 km northwest of Whitestown.

The West Lafayette station has had an 8 A.M. observation time since 1953. The method of adjustment for each monthly mean daily maximum and minimum temperature is summarized below for the two periods of record, before and after the change in equipment and the time of observation at Whitestown on 1 September 1986:

(1) 1 July 1967 through 31 August 1986:

Whitestown: 7 A.M. observation time; liquid-in-glass thermometers, Cotton Region Shelter.

West Lafayette 6NW: 8 A.M. observation time; liquid-in-glass thermometers, Cotton Region Shelter.

(2) 1 September 1986 through December 1993:

Whitestown: 5 P.M. observation time; Maximum-Minimum Temperature System.

West Lafayette 6NW: 8 A.M. observation time; liquid-in-glass thermometer, Cotton Region Shelter.

The method for computing the Whitestown (WHT) adjustments for each monthly mean daily maximum and minimum temperature from the homogeneous West Lafayette 6NW (WLF) record was:

$WLF_{1\text{-}2} = WLF_1 - WLF_2$ = Assumed climate change from period 1 to period 2 at both West Lafayette (WLF) and Whitestown (WHT; not shown in this equation).

$WHT_{1\text{-}2} = WHT_1 - WHT_2$ = Climate change + non-climatic change caused by changes in observation time and instrumentation at Whitestown.

Temperature adjustment at Whitestown 1 Sept. 1986 to 31 Dec. 1993 $= WHT_{1\text{-}2} - WLF_{1\text{-}2}$ = (Climate + Observation change) - Climate change

These estimated adjustments (Table 1) were applied to the September 1986 through December 1993 records from Whitestown to create a homogeneous 7 A.M. series. Since the airport temperature series is based on midnight observations, the Whitestown 7 A.M. series (1 January 1955 to 31 December 1993) was then adjusted (see Karl, *et al.,* 1986) to a midnight observation base with the time of observation bias adjustments given for the nearest 7 A.M. stations (Kokomo 7SE, Greencastle 1E, and Elwood) provided in the Indiana Monthly Station Normals (National Climatic Data Center, 1992). No adjustments were made to the airport records.

The airport and adjusted Whitestown data series (1955-1993) were used to regress January, July, and mean daily maximum and minimum temperatures on year. The differences between each of the data series were also regressed on year. A simple linear regression program was used with the years coded by their last two digits (55, 56,....., 93) to minimize roundoff errors. The slope of the regression line for the temperature differences between the airport and Whitestown on year should reveal any linear trends in the urban heat island effect at the airport over the last 39 years, assuming that the heat island effect does not reach Whitestown.

Using the 1961-1990 National Climatic Data Center (1992) normals, the 30-km north-south temperature gradients between the airport and Whitestown for the mean daily maximum and minimum temperatures for all months and the year were compared with those calculated for an average 30-km north-south temperature gradient in western and central Indiana. Three pairs of rural climatological stations having 30-year normals and time of observation bias adjustments were used to define the average rural north-south temperature gradient: Terre Haute 8S to Perrysville 4WNW; Greencastle 1E to West Lafayette 6NW; and

Table 1. Adjustments to published (U.S. Weather Bureau from 1909 to June 1965 and the U.S. Department of Commerce from July 1965 to 1993) mean daily maximum and minimum temperatures (°F) for Whitestown, Indiana, used to obtain a 7 A.M. observation day base for the indicated month and years as well as adjustments (National Climatic Data Center, 1992) to the 7:00 A.M. series (*) to obtain a series with a midnight (MID) observation base.

	Adjustments For							
	Mean Daily Maximum Temperature To Convert To A 7:00 A.M. Observation Base				Mean Daily Minimum Temperature To Convert To A 7:00 A.M. Observation Base			
Month	1/1/09[1] - 9/30/65	10/1/65 - 8/31/86	9/1/86 - Present	7 A.M. to MID Base*	1/1/09[1] - 9/30/65	10/1/65 - 8/31/86	9/1/86 - Present	7 A.M. to MID Base*
January	- 0.6	0	- 0.9	0.2	- 2.5	0	- 3.1	1.2
February	- 1.2	0	- 2.0	0.4	- 2.5	0	- 3.7	1.7
March	- 1.9	0	- 2.7	0.4	- 1.7	0	- 3.2	1.1
April	- 1.3	0	- 1.8	0.4	- 1.5	0	- 2.7	0.0
May	- 1.1	0	- 0.9	0.3	- 1.0	0	- 2.0	- 0.6
June	- 0.7	0	- 1.6	0.2	- 0.5	0	- 1.1	- 0.5
July	- 0.2	0	- 1.5	0.0	- 0.5	0	- 1.0	- 0.4
August	0.0	0	- 1.2	0.0	- 1.0	0	- 2.0	- 0.3
September	0.2	0	- 1.0	0.0	- 2.0	0	- 2.6	- 0.4
October	- 0.3	0	- 1.6	- 0.1	- 0.9	0	- 2.9	0.5
November	- 0.6	0	- 1.3	0.0	- 1.4	0	- 3.0	1.0
December	- 0.4	0	- 0.4	0.0	- 1.7	0	- 2.5	0.7
Year	- 0.7	0	- 1.4	0.2	- 1.4	0	- 2.5	0.3

[1] Epperson and Dale, 1984

Greenfield to Elwood. The locations of these station pairs are shown on Figure 1. Higher elevations in eastern Indiana make the north-south temperature gradients there less representative of the airport to Whitestown area. All station normals were adjusted to a midnight base using the time of observation bias estimates provided with the National Climatic Data Center 30-year normals.

The difference between the airport to Whitestown north-south temperature gradient and the average north-south gradient for the three rural station pairs for the mean daily maximum and minimum temperatures for each month were used to estimate temperature adjustments to the airport data to provide more representative "rural" temperatures. Heating degree day normals for each month at the airport were then computed from the airport's adjusted mean daily temperature using the rational method developed by Thom (1954) for truncated (at the 65° F base) normal distributions:

$$N\,\bar{D} = N(65 - \bar{T} - l\,s_{\bar{T}}\sqrt{N})$$

where N is the number of days in the month, $\bar{D}$ is the daily mean heating degree days for the month, 65° F is the base of the heating degree days, $\bar{T}$ is the

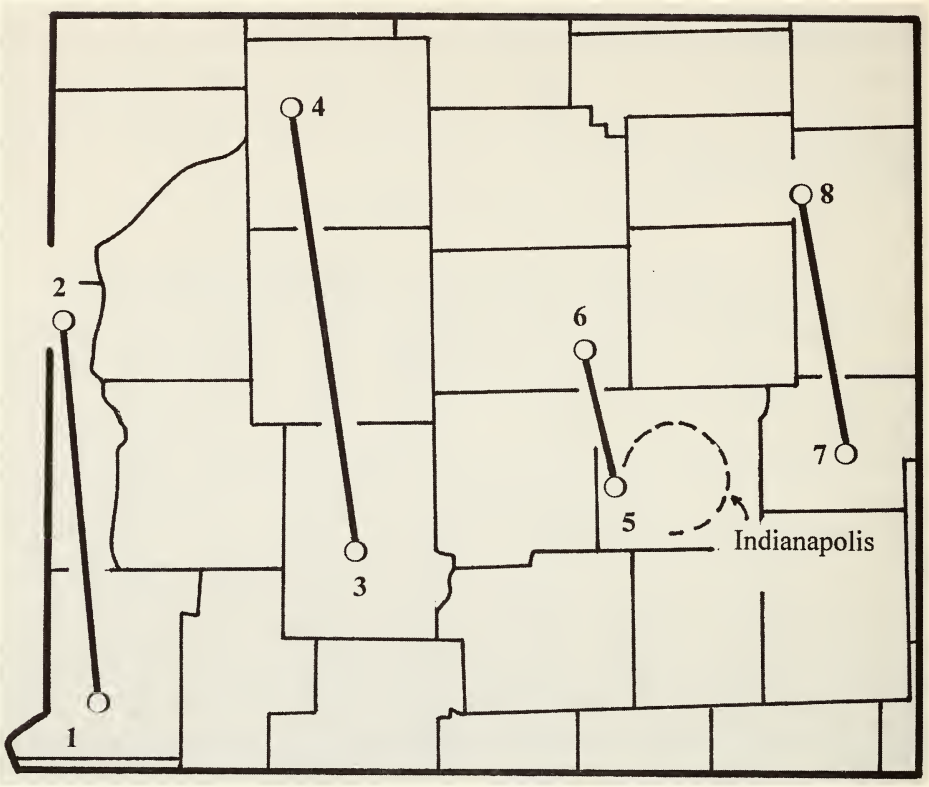

Figure 1. The location of the climatological stations with 30-yr normals used to determine the rural north-south temperature gradient adjustments to Indianapolis International Airport temperatures (1 = Terre Haute 8S; 2 = Perrysville 4WNW; 3 = Greencastle 1E; 4 = West Lafayette 6NW; 5 = Indianapolis International Airport; 6 = Whitestown; 7 = Greenfield; and 8 = Elwood). The major urban area of Indianapolis is shown by the dashed circle.

mean temperature estimated as the mean of the daily mean maximum and minimum temperatures, l is an empirical function of h (see Table 1 in Thom (1954)), h is calculated as $(65 - \bar{T})/s_{\bar{T}} \sqrt{N}$, and $s_{\bar{T}}$ is the standard deviation of the mean monthly temperature as interpolated from the National Atlas Charts (Thom, 1955).

RESULTS AND DISCUSSION

The *a priori* temperature adjustments made to the Whitestown control station temperatures before comparison with the Indianapolis Airport's temperatures are shown in Table 1. For example, in March after 1 September 1986 (5 P.M. observation time), 2.7° F must be subtracted from the published mean daily maximum and 3.2° F from the daily mean minimum temperatures before comparing them with those from the same station when it had a 7 A.M. observation time (from October 1965 through August 1986). In addition, for temperatures before October 1965 (6 P.M. observation time), 1.9° F must be subtracted

from the March daily mean maximum and 1.7° F from the mean daily minimum temperatures before comparing them with those in the 1965-1986 time period. (Note that these two time of observation bias adjustments for 5 P.M. (since September 1986) and 6 P.M. (before 1965) signal the discrepancy in "climate" which occurs in areas which go on Daylight Savings Time from April through October, unless the observer changes his time of observation (say from 5 P.M. EST to 6 P.M. EST).) Finally, to compare the new 7 A.M. adjusted mean temperature series to the midnight-based airport mean temperatures, 0.4° F must be added to the March mean daily maximum at Whitestown and 1.1° F to the mean daily minimum.

Regression analysis of the airport and Whitestown temperature variables for January, July, and annual on year (1955-1993) are summarized in Table 2. Of the 12 regressions, the only significant time trend ($\alpha = 0.05$) was the airport's annual mean daily minimum temperature with a trend of 0.04° F (± 0.02)/year or an estimated warming of 1.5° F in the last 39 years.

Two significant trends emerged in the six regressions of the airport to Whitestown gradient on year, the July, and annual mean daily maximum temperatures. The July average daily mean maximum temperature differences have increased 0.04° F (± 0.01)/yr or 1.6° F in the last 39 years. July, of course, does not affect the heating degree days but does affect cooling degree days (not discussed here). The annual mean daily maximum temperature difference has increased an average of 0.02° F (± 0.01)/yr or 0.8° F in the last 39 years. No significant time trends were found in the airport to Whitestown differences for the January mean daily maximum and minimum temperatures or for the annual mean daily minimum temperatures (Table 2), but the annual mean daily minimum temperature at the airport averaged 2.8° F (± 0.8) above that at Whitestown. Since any climatic change should be reflected in the temperature at both the airport and Whitestown, these significant trends in the temperature differences suggest increased urban heat island and/or increased upwind runway pavement area effects at the airport.

The comparison of the north-south temperature gradients between the airport and Whitestown with the averaged gradients from the three rural station pairs for the 30-year normals (1961-1990), adjusted to a midnight observation base, are shown in Table 3. The standard deviations are also included to show that the average gradients were usually beyond two standard deviations. For example, in March (Table 3), the airport to Whitestown gradient is 1.4° F for the mean daily maximum and 2.6° F for the mean daily minimum temperatures compared to the rural average of 1.0 ± 0.2 and 1.0 ± 0.4, respectfully, resulting in a - 0.4° F adjustment to the airport's March mean daily maximum, -1.6° F to the mean daily minimum, and -1.0° F to the daily mean temperature. "Rural adjustments" to the airport's mean daily maximums are minor, averaging 0.2° F for the year, but they are consistently negative for the mean daily minimums, averaging - 1.8° F and - 0.8° F for the mean. The temperature gradients in west-central and central rural Indiana averaged 0.7° F for the mean daily maximum and 0.9° F for the mean daily minimum per 30 km.

Table 2. Simple linear regression statistics for the Indianapolis International Airport, Whitestown, Indiana, and the temperature gradient between them on year, 1955-1993.

Station	Variable	Mean °F ± Std Dev	Slope ± Std Error	*r*	*F*
Indianapolis International Airport	January Mean Daily Maximum	34.2 ± 5.3	0.06 ± 0.08	0.12	0.5
	January Mean Daily Minimum	18.0 ± 5.8	0.10 ± 0.08	0.19	1.3
	July Mean Daily Maximum	85.2 ± 2.5	0.05 ± 0.04	0.24	2.3
	July Mean Daily Minimum	65.2 ± 2.1	0.04 ± 0.03	0.25	2.4
	Annual Mean Daily Maximum	62.0 ± 1.2	0.03 ± 0.02	0.25	2.0
	Annual Mean Daily Minimum	42.5 ± 1.3	0.04 ± 0.02	0.34	4.8*
Whitestown	January Mean Daily Maximum	32.8 ± 4.9	0.04 ± 0.07	0.08	0.3
	January Mean Daily Minimum	16.1 ± 6.3	0.14 ± 0.09	0.25	2.4
	July Mean Daily Maximum	85.3 ± 2.4	0.01 ± 0.03	0.04	0.1
	July Mean Daily Minimum	61.7 ± 2.0	0.05 ± 0.03	0.29	3.4
	Annual Mean Daily Maximum	61.5 ± 1.3	0.01 ± 0.02	0.06	0.2
	Annual Mean Daily Minimum	39.7 ± 1.2	0.03 ± 0.02	0.28	3.1
Airport to Whitestown Gradient	January Mean Daily Maximum	1.4 ± 1.1	0.02 ± 0.02	0.19	1.4
	January Mean Daily Minimum	1.9 ± 1.7	-0.04 ± 0.02	-0.28	3.1
	July Mean Daily Maximum	0.0 ± 1.0	0.04 ± 0.01	0.53	14.6**
	July Mean Daily Minimum	3.5 ± 1.3	-0.01 ± 0.02	-0.07	0.2
	Annual Mean Daily Maximum	0.5 ± 0.6	0.02 ± 0.01	0.35	5.2*
	Annual Mean Daily Minimum	2.8 ± 0.8	0.01 ± 0.01	0.14	0.7

* $F_{0.05\,[1,\,37]} = 4.11$.
** $F_{0.01\,[1,\,37]} = 7.37$.
r = Correlation coefficient.
F = Calculated value of *F*.

The calculated adjustments for a rural environment to the monthly and annual heating degree days at the airport are shown in Table 4. The adjustments to the daily mean temperature (the last column in Table 3) have been reproduced in the third column of Table 4 to obtain the rural gradient adjusted mean daily temperature normals. The *l* column in Table 4 is a measure of the truncation of the normal distribution of the daily mean temperatures due to the transition of daily mean temperatures across the 65° F threshold of the heating degree days. For example, in January, February, March, November, and December, the mean is sufficiently below the 65° F bound that no daily temperatures within the month are above 65° F, and *l* is 0. In other months, however, *l* is not 0, and the truncated portion, $l\, s_{\bar{T}}\sqrt{N}$, has to be added to the 65 - $\bar{T}$ component. Our results showed

Table 3. Average 30-km north-south temperature gradients (°F, 1961-1990 normals) between the Indianapolis International Airport and Whitestown, Indiana, and the mean gradients with their standard deviations for three rural station pairs in western and central Indiana (Terre Haute 8S to Perrysville 4WNW; Greencastle 1E to West Lafayette 6NW; and Greenfield to Elwood) with the estimated rural temperature adjustments for the airport. All normals were adjusted to a midnight observation base. The 30-yr normals from 1961 to 1990 for Whitestown were calculated from the same adjusted temperature series used in the airport to Whitestown regressions, but the normals used for the other 6 (3 pairs) of rural stations were obtained from the National Climatic Data Center (1992).

	Mean Rural Gradient		Airport to Whitestown Gradient		Adjustments To The Airport For A Rural Environment		
Month	Mean Daily Maximum ± Std Dev	Mean Daily Minimum ± Std Dev	Maximum	Minimum	Maximum	Minimum	Mean
January	0.9 ± 0.1	0.9 ± 0.3	1.3	1.9	- 0.4	- 1.0	- 0.7
February	1.1 ± 0.2	1.0 ± 0.4	0.9	2.3	0.2	- 1.3	- 0.7
March	1.0 ± 0.2	1.0 ± 0.4	1.4	2.6	- 0.4	- 1.6	- 1.0
April	0.7 ± 0.3	1.0 ± 0.2	0.7	3.1	0.0	- 2.1	- 1.0
May	0.6 ± 0.3	1.0 ± 0.3	0.1	3.3	0.5	- 2.3	- 0.9
June	0.5 ± 0.3	0.8 ± 0.4	- 0.1	3.1	0.6	- 2.3	- 0.8
July	0.6 ± 0.2	0.9 ± 0.4	- 0.1	3.5	0.7	- 2.6	- 1.0
August	0.7 ± 0.3	1.1 ± 0.3	0.0	3.7	0.7	- 2.6	- 1.0
September	0.5 ± 0.3	1.1 ± 0.3	- 0.4	3.9	0.9	- 2.8	- 1.0
October	0.6 ± 0.2	1.0 ± 0.2	0.3	2.3	0.3	- 1.3	- 0.5
November	0.6 ± 0.2	0.8 ± 0.4	0.9	1.6	- 0.3	- 0.8	- 0.6
December	0.8 ± 0.1	0.7 ± 0.3	1.2	2.0	- 0.4	- 1.3	- 0.8
Annual	0.7	0.9	0.5	2.8	0.2	- 1.8	- 0.8

that the annual total of the monthly heating degree day adjustments is 251, or about 4% above the published heating degree day annual normal of 5615 for the airport. Grant (personal communication, R.H. Grant, Agronomy Department, Purdue University, 5 July 1994) compared the seasonal heating degree day total computed with Thom's method with those computed from the actual daily mean temperatures for the airport (1955-1993) to find that Thom's method underestimated the seasonal heating degree days by about 50 heating degree days. This additive methodological bias brings the estimated seasonal heating degree day bias to 301 (about 5% too low).

We do not know whether or not this heating degree day adjustment will create any significant changes in the methods of estimating total gas use during the heating season in Indiana. Also, some segments of the heating industry use population-weighted heating degree days for estimating fuel use. Perhaps unknow-

Table 4. Calculated adjustments to the heating degree day (HDD) normals for the Indianapolis International Airport with adjusted mean "rural" temperature normals from 1961 to 1990.

	Mean Daily Temperature (F)							
Month	Present Normal	"Rural" Temp. Adjust.	Adjusted Rural Normal	Standard Error[1] S_T	l^2	"Rural"[3] HDD Normal	Present HDD Normal	"Rural" HDD Adjusted
January	25.5	- 0.7	24.8	5.7	0	1246	1225	21
February	29.6	- 0.7	28.9	5.0	0	1011	991	20
March	41.4	- 1.0	40.4	5.3	0	763	732	31
April	52.4	- 1.0	51.4	3.2	0.01	413	378	35
May	62.8	- 0.9	61.9	3.6	0.13	177	165	12
June	71.9	- 0.8	71.1	3.2	0.41	33	5	28
July	75.4	- 1.0	74.4	2.6	0.66	5	0	5
August	73.2	- 1.0	72.2	2.7	0.51	15	6	9
September	66.6	- 1.0	65.6	3.3	0.21	96	58	38
October	54.7	- 0.5	54.2	3.5	0.02	347	338	9
November	43.0	- 0.6	42.4	3.5	0	678	660	18
December	30.9	- 0.8	30.1	4.8	0	1082	1057	25
Annual	52.3	- 0.8	51.5			5866	5615	25

[1] From the isoline charts of Thom (1955).
[2] l = f(h), Table 1 in Thom (1954); $h = (65 - \overline{T})/ {}^{s}\overline{T}\sqrt{N}$.
[3] Monthly heating degree days = $N\overline{D} = N(65 - \overline{T} + l^{s}\overline{T}\sqrt{N})$.

ingly, the airport heating degree day bias may provide something of a population-weighted heating degree day prediction! These questions are for the heating industry to answer.

CONCLUSIONS

The urban heat island effect at the Indianapolis International Airport is small but measurable. The bias is found primarily in the mean daily minimum temperature, which for the year averages about 1.8° F higher than would be expected in a more rural setting. Also, of the 12 temperature regressions on year examined, only the airport's annual mean daily minimum had a significant (α= 0.05) upward time trend, possibly due to the greater frequency of urban air pollution and heat backing to the airport under the typically calmer conditions of the night and early morning hours. The temperature differences between the airport and Whitestown showed the greatest upward time trends for the annual and July mean daily maximum temperatures, the only significant time trends in the six gradient variables. These trends may be caused by the increased mass of run-

way pavement upwind of the hygrothermometer. The mean temperature for the year at the airport was estimated as 0.8° F above that of a "rural" setting. This difference produces a downward bias in the seasonal heating degree days (base 65° F) of about 251 heating degree days for the year. If we add to this the 50 seasonal heating degree day bias found by Grant (personal communication, 1994), the total bias becomes 301 or about 5% of the published seasonal heating degree day normals for the airport.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 319-325

NOTES ON A WINTER COLONY OF BIG BROWN BATS AT WILLIAMSPORT, WARREN COUNTY, INDIANA

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ABSTRACT: A single hibernaculum for big brown bats (*Eptesicus fuscus*) in the attic of a large old apartment building was studied over five winters. The number of bats generally increased during a period characterized by mild winters during which temperatures in the attic apparently always remained above freezing. However, many bats left during a very cold period in the late winter of 1993, when at least part of the attic reached temperatures below freezing. Turnover in the population using the attic was high both among years and within years. The bats spread out over the available area with essentially no clustering. The bats had a tendency to return to their favorite sections of the hibernaculum, but individuals almost never returned to the exact same spot. Sex ratios varied from 50.0% to 74.2% males. Several bats spent both the winter and summer in this roost.

KEYWORDS: Bats, big brown bat, Chiroptera, *Eptesicus fuscus*, hibernation.

INTRODUCTION

Whitaker and Gummer (1992) reported on big brown bats (*Eptesicus fuscus*) in winter and concluded that, although some hibernate in caves and mines, the majority of the big brown bats in Indiana hibernate in buildings that maintain temperatures above freezing throughout the winter. Some of the buildings also support summer colonies, but most that serve as hibernacula lack summer colonies. Most of the buildings that serve as hibernacula in winter harbor only a few bats (1-10), but a few harbor as many as 70 or 80.

The purpose of this study was to evaluate population size, site fidelity, sex ratio, and summer use by big brown bats that hibernated in one specific hibernaculum, the attic of an old brick apartment building in Williamsport, Warren County, Indiana. In summer, this building contains a maternity colony of slightly over 200 individuals. A number of questions were asked about this colony of big brown bats:

1. Does the number of bats using the building for hibernation change between and within years?
2. How much turnover occurs between and within years?
3. How many bats are present in one year but not the next?

4. Do the bats go back to the same spot or the same general location each year?
5. Is the sex ratio similar among and between years?
6. Do hibernating bats also spend the summer there?

MATERIALS AND METHODS

The approach taken during this study was to examine the entire attic at Williamsport from 1 to 4 times per winter over 5 winters (1989-1993). The attic is big, about 40′ x 60′, but the ceiling is low (from 3 to 10 feet high), and few hiding places for bats are present. Therefore, nearly all of the bats could be captured during each examination. The bats generally hung from the rafters, although a few bats were nearly always found in the cracks between the beams. The attic was separated by partitions into five sections. On each visit to the attic, each bat was caught and weighed, its sex was noted, and it was either banded or its band number was noted.

The attic was first visited on 17 January 1989 (winter 1), and 22 bats were found. All were banded with small, white plastic bands. The colony was visited five times the following winter (winter 2) between 10 January and 6 April 1990. Some bats had removed their white bands, so starting in 1989-90 (winter 3), larger yellow plastic bands were used. A few bats chewed these off. Therefore, starting in 1991-92 (winter 4), metal bands were used. Old bands were left on individuals previously captured. Loss of bands from multiple-banded bats thus gave us a measure of the rate of disappearance of bands. In 1990-91 (winter 3), only one trip was made (19 December); one was made in winter 4 (31 December 1992); and 2 were made in winter 5 (2 January and 26 February 1993).

RESULTS

In the beginning, I assumed that some bats probably returned to the same hibernacula and perhaps even to the same hibernating spot each year. To test whether bats returned to the same spot in consecutive years, a numbered and dated tag was pinned on the building below each bat as we banded it during the first year of the study. However, after completing the second year's observations here and in other colonies, almost no bats were found in the same spot where they had been banded the year before, and we stopped putting up these tags. Favored spots, such as particular cracks, almost always contained hibernating bats. It was in these few favored places that the same individuals were found during more than one year. In general, bats simply spread out over the available space. In addition, the bats moved between different hibernacula within and between years (as evidenced by bats being present one year, gone the next, and returning later). Thus, repeated hibernation at the same spot in consecutive years by the same bat was the exception, certainly not the rule.

Although few bats return to the exact same place in consecutive years, I also examined whether they returned to the same general area. The attic had five separate sections which were used to assess whether the bats returned to the same

general vicinity each year. Twenty bats recorded more than once were always found in the same section – 1 for 4 years, 4 bats for 3 years, and 15 bats for 2 years (12 in consecutive years, and 3 in 2 years separated by one year when the bat was not seen at all). This finding seems to show a slight tendency by the bats to return to favored sections of the attic, and significantly more bats returned to the same section than would be expected by chance ($x^2 = 35.6$, $df = 1$). On the other hand, 3 bats occurred at different times in 3 separate sections, and 32 bats were found in two separate sections.

Although the number of samples taken differ, the high number of bats per visit for the five years were 22, 32, 33, 43, and 63. The number of bats wintering in this roost showed a significant increase during the study period ($x^2 = 25.2$, $df = 4$). The increase may have occurred because this study was done over a series of relatively warm winters. Within individual years, change also occurred. In 1989-90, 19 bats were present on 10 January, 31 on 2 February, 24 on 9 March, and 54 on 20 March. In 1992-93, 63 were present on 2 January (there had not been much cold weather to this date), but only 20 on 26 February. This latter date was at the end of a very cold period, and frost formed on the inside of the roof, especially in section 1. No bats were found in section 1 at this time, although bats were present here during every other visit.

No bat was recorded during every visit or during each year. Only one bat was recorded in four of the five years, and only 5 bats were seen in three of the five years. One bat was seen on six visits over 3 years, and one was seen on six visits over 2 years. One other bat was seen on five visits, and 5 were seen on 4 visits.

Obviously, the number of bats in the building's attic fluctuated both between years and within years (Table 1). Twenty-two bats were banded on 19 January in winter 1 (Table 1). Only three of these were seen the next year. One, a male, was seen on all five visits in 1989-90 but was never seen again. One bat was present in winter 3 but not winter 4, and another was present in winter 3 and winter 4.

The roost was visited five times in 1989-90, and a total of 94 different bats was seen, including the 3 bats banded in 1988-89. Of the 94, 53 were present on only one date, and 13 were present twice. Of those present only one time, 4 were present only on the first date, 10 were present only on the second date, 7 were present only on the third date, and 32 were present only on the fourth date. All nine bats taken on the last date that year had been previously banded. Fourteen bats were present twice (including those banded the previous year) in all different combinations (visits 1 and 2 = 1 bat; 1 and 3 = 2; 1 and 4 = 1; 1 and 5 = 1; 2 and 3 = 2; 2 and 4 = 2; 2 and 5 = 1; 3 and 4 = 2; and 3 and 5 = 1). Those present 3 times were one bat on visits 1, 4, 5; 1 on 1, 2, and 4; 1 on 1, 3, and 4; and 1 on 2, 4, and 5. Three were present on 4 visits, two of them on the first 4, and one on the first two and the last two visits.

In 1992-93 (year 5), the roost was visited twice. The total number of bats seen during the first visit was 64, of which 43 had not been previously banded.

Table 1. Recurrence of individual big brown bats in an attic in Williamsport, Indiana. (For example, of the 22 big brown bats captured on 19 January 1989, 19 were never seen again, 1 was seen on the next 5 visits, 1 was seen on 3 later visits, and 1 was seen on 4 later visits.)

Number of Bats	Sex M	Sex F	Winter 1 1988-89 17 Jan.	10 Jan.	2 Feb.	Winter 2 1989-90 9 Mar.	20 Mar.	6 Apr.	Winter 3 1990-91 19 Dec.	Winter 4 1991-92 31 Dec.	Winter 5 1992-93 2 Jan.	26 Feb.
Totals:			22	19	32	24	54	9	34	44	64	20
19	8	11	●									
1	1		●	●	●	●	●	●				
1		1	●		●	●			●			
1	1		●			●		●	●	●		
4	1	3		●								
10	8	2			●							
7	4	3				●						
32	13	19					●					
1		1		●	●							
1	1			●		●						
2	1	1		●			●					
1	1			●	●	●	●					
1	1			●				●				
1	1			●			●	●				
1	1			●	●		●	●				
2	2			●	●		●		●			
1		1		●						●		
1		1		●		●			●		●	
1	1			●		●	●			●		●
5	5				●	●						
1	1				●		●					
1	1				●			●				
2	1	1			●		●					
1		1			●				●			
1	1				●		●		●			
1	1				●				●		●	
1		1			●	●					●	
1	1				●				●	●	●	
1	1					●	●					
1	1					●	●		●			
1		1				●				●		
1		1					●		●			
1	1						●			●		
2	2						●		●		●	
2	1	1					●			●	●	
1	1							●				
13	9	4							●			
4	4								●	●		
2	1	1							●		●	
1	1								●	●	●	
21	13	8								●		
8	7	1								●	●	
1		1								●		●
1		1								●	●	●
33	26	7									●	
10	6	4									●	●
7	4	3										●

Of the 21 that had been previously banded, 9 had been banded in year 2, 3 in year 3, and 9 on 31 December of the previous year. Other than those banded in the previous visit and one banded on 19 December 1990 (which was seen for 3 consecutive visits and 3 consecutive winters), none had been observed on every visit since the bat was first banded. Only one of the 9 bats banded in year 2 was observed in each year afterward. Some bands were being removed by the bats. One bat was given a white band on 19 January 1989 and a yellow band on 10 January 1990. However, by 20 March, this bat had lost the yellow band but retained the white one (one of two instances when yellow bands were lost).

New (unbanded) bats were constantly showing up throughout the study. The new arrivals coupled with the many absentees who later reappeared was convincing evidence that many bats moved between buildings throughout the winter.

Clustering of Bats. As Whitaker and Gummer (1992) discovered, little clustering occurred. The bats spread out over the available area. For example, of the 64 bats at Williamsport on 2 January 1993, all were solitary except for two pairs.

Sex Ratios. Winter sex ratios ranged from 50.0% to 74.2% males over the course of the study, varying somewhat both within and between years.

Relation Between Summer and Winter Use of the Buildings. Since the numbers of bats are much smaller in buildings in winter, even in buildings used both in winter and summer, the great majority of bats obviously hibernate elsewhere than in their summer roost. Since no winter roost has very many bats in winter (most less than 5), the bats from one roost apparently spread out and hibernate in a large number of buildings. One visit was made in the summer to determine the relationship of the summer to the winter bats. A total of 133 bats had been banded there in winter by that time (55 females and 78 males). On 19 December 1990, thirty-three bats were hibernating at Williamsport (24 males and 9 females). In the following summer (13 August 1991), 36 bats banded in previous years were present in the maternity colony (out of an estimated total of 280 bats). Most of the adults were assumed to be females, and about half of the bats were assumed to be young of the year. Therefore, about 36 of the 140 adult bats present were banded. Obviously, many of the bats had hibernated elsewhere, but a number of bats spent both the winter and summer in the hotel.

DISCUSSION

Big brown bats are essentially solitary in winter, each finding its own individual hibernating spot. The hibernaculum must remain above freezing throughout the winter. Big brown bats were found to move between hibernacula in winter in this and in other colonies. No apparent reason existed for their movement in and out of the hibernaculum as no particularly cold spells occurred during this period. Movement in and out of the hibernaculum may represent normal behavior for this species in winter. Whitaker and Gummer (1992) postulated that movement between hibernacula is beneficial to the bats if the movement makes them aware of additional hibernacula in the area that could be used in the event

of the loss of one through fire or from human intervention (building destruction, exclusion, turning off the heat, and so forth). The behavior of the big brown bat is very different from the behavior of some other species that hibernate in caves. For example, when hibernating in caves, the individuals of *Myotis lucifugus* and *M. sodalis* often congregate in clusters and show great fidelity to the hibernaculum, although some winter movement between caves does occur (Barbour and Davis, 1969; Fenton, 1966; Hall, 1962; Hardin and Hassell, 1970; Humphrey and Cope, 1976; Griffin, 1940; LaVal and LaVal, 1980). Because they are permanent, caves are relatively safe, and they provide a stable environment. Thus, returning to the same cave each year makes good evolutionary sense, although some cave switching does occur.

Big brown bats, on the other hand, have adapted to man's structures (especially to barns but also to churches, houses, and other buildings in the summer). Most of the buildings used in the summer are too cold to be used as winter hibernacula. Buildings, especially heated older ones like the apartment building the bats use in Williamsport, are particularly vulnerable to destruction or loss of ancillary heating. Therefore, the bats may need to find a new hibernaculum at any time, including midwinter. Big brown bats tend to be solitary in winter, but they are probably aware of additional hibernacula in the area in case they need to depart abruptly. Most hibernacula have low numbers of bats. A winter hibernaculum that has remained above freezing for a number of years may attract an increasing numbers of bats, because enough time has elapsed for the hibernaculum to be discovered by more and more individuals. The original hibernacula of big brown bats were probably trees. Like buildings, trees are also vulnerable to destruction; they can burn, blow over, or die. Perhaps, the bats that now use buildings as hibernacula originally used trees. Since the same risks would have applied to tree loss, the big brown bats were probably solitary there as well.

How does the Williamsport colony relate to these ideas? Williamsport has a large summer colony (several hundred bats), but only a small proportion of them use the apartment building's attic as a winter hibernaculum. The attic was available every year of the study, and the numbers in winter had been increasing, although a big decrease occurred on 26 February 1993, when only 20 bats were found. The increase was related to the weather, which was characterized by relatively warm winters during the study period. No exceptionally long cold periods (when the air in the attic would drop below freezing for more than a day or two) occurred in the first four winters of the study; thus, Williamsport remained a viable hibernaculum, accounting for the increase in numbers. However, in February 1993, an extended very cold spell occurred in which the temperature dropped far below freezing. The coldest part of the attic at Williamsport is section 1. On February 26, frost was found on the ceiling of section 1, and no bats were found there. Fewer bats were present in the other sections of the attic when compared to 2 January count (64 in comparison to 20). The cold caused many of the bats to leave the hibernaculum. They did not move downstairs or

anywhere else in the building, because no other space was available to them in the building.

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Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 327

THE MASSASAUGA IN INDIANA

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In my Indiana records, I have a number of specimens of the eastern massasauga (*Sistrurus catenatus catenatus*) from Wabash and Fulton Counties:

1. Fulton County on 30 April 1970 near Akron: A specimen 25½ inches long was found in a marshy bog by Frank Bynum, a biology teacher at Akron High School. The specimen was deposited in their biology lab. Since that time, Akron High School has become part of Tippecanoe Valley High School. The exact status of the specimen is unknown.
2. Wabash County on 19 April 1970 near North Manchester: A specimen 22 inches long was collected in a marshy bog by the author. This specimen was deposited at the University of St. Francis in Fort Wayne.
3. Wabash County on 1 September 1970 near North Manchester: A snake 22½ inches long was collected in a wetlands woods by the author. The specimen is with James D. Shull, a biology teacher at Homestead High School in Fort Wayne.
4. Wabash County on 30 April 1978 near North Manchester: A melanic male 31½ inches in length was collected by the author when the snake was hiding between a raspberry patch and a big shade tree. This specimen is in the author's personal collection.

These four specimens are preserved in formaldehyde. In addition to these specimens, two recent sight records of the eastern massasauga are know to the author. The author saw the eastern massasauga in April 1995 in a wetlands area in Steuben County. The second sighting was made by John Shuey in mid-summer 1998 in a prairie habitat in LaGrange County.

Proceedings of the Indiana Academy of Science
(1997) Volume 106 p. 329-332

THE REDISCOVERY OF *LATHYRUS OCHROLEUCUS* (LEGUMINOSAE) IN INDIANA

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ABSTRACT: The pale vetchling peavine (*Lathyrus ochroleucus*) has not been reported in Indiana since the late 1930's. Field reconnaissance by the Indiana Department of Natural Resources prior to 1993 located no live specimens of *L. ochroleucus* at the locations recorded by earlier botanists. *Lathyrus ochroleucus* was subsequently listed as extirpated in Indiana. In 1994, two small populations of *L. ochroleucus* were discovered growing in rural Steuben County, Indiana. These populations are thriving and reproducing. *Lathyrus ochroleucus* is worthy of being upgraded to endangered status in Indiana.

KEY WORDS: Extirpated, *Lathyrus ochroleucus*, rare plants, Steuben County, Indiana.

INTRODUCTION

Lathyrus ochroleucus Hook., pale vetchling peavine, is listed as an extirpated vascular plant species in Indiana (Indiana Department of Natural Resources, 1993). A rhizomatous, perennial herb, *L. ochroleucus* is a trailing or climbing vine with cream-colored, pea-like inflorescences. In the *Flora of Indiana*, Deam (1940, p. 618) described the habitat frequented by the species as follows: "Infrequent to rare in dry soil in black and white oak woods in the northern [Indiana] counties." In *Plants of the Chicago Region*, Swink and Wilhelm (1994, p. 443) note: "This species is rare in our area except in the timbered portions of the hill country in our northern sector. It usually occurs in the thin soil of dry oak woods, probably the quintessential vetchling of closed savannas northward."

The natural range of *Lathyrus ochroleucus* is from Quebec west to Mackenzie, British Columbia, and south to New Jersey, Illinois, Iowa, South Dakota, Wyoming, and Washington (Britton and Brown, 1970; Gleason and Conquist, 1991). The pale vetchling peavine reaches its southern limit in northern Indiana and is found in three States adjacent to Indiana. The plant is found along the Indiana border in Berrian, St. Joseph, and Hillsdale Counties in Michigan and northward into the Upper Peninsula (Voss, 1985). Swink and Wilhelm (1994) report that they have seen voucher specimens of *L. ochroleucus* from Berrian County in Michigan, Lake and McHenry Counties in northeastern Illinois, and Racine and Walworth Counties in southeastern Wisconsin. The vetchling is also reported in the literature (Swink and Wilhelm,1994) from Cook, DuPage, and Kane Counties in northeastern Illinois and from Porter and LaPorte Counties in northwestern Indiana (the authors have not seen voucher specimens from these

locations). The state-threatened *L. ochroleucus* is listed only from Williams and Lucus Counties in Ohio (Ohio Department of Natural Resources, 1984).

Historical records show that *Lathyrus ochroleucus* once lived in Porter, LaPorte, Kosciusko, Noble, DeKalb, LaGrange, and Steuben Counties in northern Indiana (Deam, 1940). Initially, *L. ochroleucus* was listed as a threatened plant species in Indiana (Bacone and Hedge, 1980). Since the plant had not been reported in Indiana since the late 1930's and had not been located during extensive field reconnaissance by the Indiana Department of Natural Resources in the early 1990's, *L. ochroleucus* was ultimately listed as extirpated in Indiana (Indiana Department of Natural Resources, 1993; Homoya, pers. comm). A random search by the senior author near his home in Steuben County, Indiana, revealed two small populations of *Lathyrus ochroleucus* in bloom in mid-May 1994.

The genus *Lathyrus* (Leguminosae) is closely related to the genus *Vicia* (Deam, 1940; Voss, 1985; Gleason and Conquist, 1991; Swink and Wilhelm, 1994). Both genera are composed of herbaceous, trailing or climbing vines with pea-like inflorescences and even-pinnate leaves ending in a tendril (Deam, 1940). In the field, the two genera are easily separated because *Lathyrus* has larger flowers, fewer and larger leaves, and larger stipules than *Vicia* (Newcomb, 1977). In addition, *Lathyrus* is pubescent along the entire length of its style, whereas *Vicia* is pubescent only at the summit (Deam, 1940; Voss, 1985). Within the genus, *Lathyrus ochroleucus* is distinguished from the other species by having: 1) more than two, ovate, glabrous leaflets (18-28 mm wide and 35-55 mm long); 2) obliquely-attached, semi-sagittate or semi-cordate, foliaceous stipules (10-20 mm broad); and 3) 2-12 flowered, peduncled, cream-colored inflorescences (Britton and Brown, 1970; Deam, 1940; Swink and Wilhelm, 1994; Voss, 1985; Gleason and Conquist, 1991). The fruit of *L. ochroleucus* is a sessile, glabrous, oblong-linear pod that is 2.5-5.0 cm in length (Britton and Brown, 1970).

A search by Indiana Department of Natural Resources, Division of Nature Preserves, of their element occurrence records indicated that Indiana botanists began to report *L. ochroleucus* in 1905 and that reports of the species ended in 1938. Several of the sites listed were revisited in 1979 and 1980. In every case, the habitat for the plant had been destroyed, and the species was not observed. Following its rediscovery in 1994, an extensive literature search, which was carried out in April 1995, revealed no other new accounts. The senior author's voucher specimen was confirmed by Michael Homoya (pers. comm.) and is deposited in the Indiana University Herbarium. An element occurrence record for *Lathyrus ochroleucus* was filed with the Indiana Department of Natural Resources, Division of Nature Preserves, by the author in June 1994.

The two small populations of *Lathyrus ochroleucus* are located on the well-drained, south-facing bank of a county road in central Steuben County, Indiana. The revegetated road cut is located within a steeply-sloped, Casco gravelly sandy loam soil unit (U.S. Department of Agriculture, 1981). Both sides of the east-west traversing road are lightly to moderately forested with mature

trees. The populations of *Lathyrus ochroleucus* are located within the edge of this dry forest community which is composed of both native and introduced vascular plant species. Canopy associate species include white oak (*Quercus alba*), red oak (*Q. rubra*), shagbark hickory (*Carya ovata*), and white ash (*Fraxinus americana*). Sub-canopy associate species include flowering dogwood (*Cornus florida*), sassafras (*Sassafras albidum*), mapleleaf viburnum (*Viburnum acerifolium*), and autumn olive (*Elaeagnus umbellata*). Herbaceous associates include but are not limited to pussy toes (*Antennaria plantaginifolia*), rue anemone (*Anemonella thalictroides*), Kentucky bluegrass (*Poa pratensis*), Canada bluegrass (*Poa compressa*), poverty oat grass (*Danthonium spicata*), Pennsylvania sedge (*Carex pensylvanica*), short-headed bracted sedge (*C. cephalophora*), smooth brome (*Bromus inermis*), heart-leaved aster (*Aster sagittifolius*), big-leaved aster (*A. macrophyllus*), round-lobed hepatica (*Hepatica americana*), woodland sunflower (*Helianthus divaricatus*), prairie phlox (*Phlox pilosa*), and Solomon's seal (*Polygonatum canaliculatum*).

Lathyrus ochroleucus bloomed for a period of approximately three weeks in middle to late May. Several of the plants produced fruit in mid to late June in both 1994 and 1995. In 1995, a high percentage of the maturing pods were consumed by white-tailed deer. The two small populations are located approximately 50 m apart at the top of the road embankment. The western plot had approximately 21 plants within an area of approximately 2.3 m^2. The eastern plot had approximately 57 plants within an area of approximately 27.0 m^2. The two populations are located within the standard 22.5-foot (6.9 meters) county-owned right-of-way but are beyond the normal limits of roadside mowers. Threats of extirpation to these populations of *Lathyrus ochroleucus* include changes in the habitat due to accumulation of oak leaf litter, shading by the exotic, invasive *Elaeagnus umbellata* sub-canopy species, browsing by the local deer population, and potential road widening. Given the location of these populations, the species may be able to withstand a limited amount of disturbance. However, open woods and savannas, the preferred habitat for *L. ochroleucus*, are declining in area across its normal range, due to development pressures and the suppression of fire (Swink and Wilhelm, 1994). Since *L. ochroleucus* has been rediscovered in Indiana, the species should be upgraded to endangered status.

ACKNOWLEDGMENTS

The authors wish to thank Mike Homoya for his encouragement as well as for his review and comments on this manuscript. Special thanks go to the senior author's grandmother, Elizabeth Felton Wilson, for instilling a desire in him to pursue amateur botany.

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PRINTED ON RECYCLED PAPER

PROCEEDINGS OF THE INDIANA ACADEMY OF SCIENCE

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